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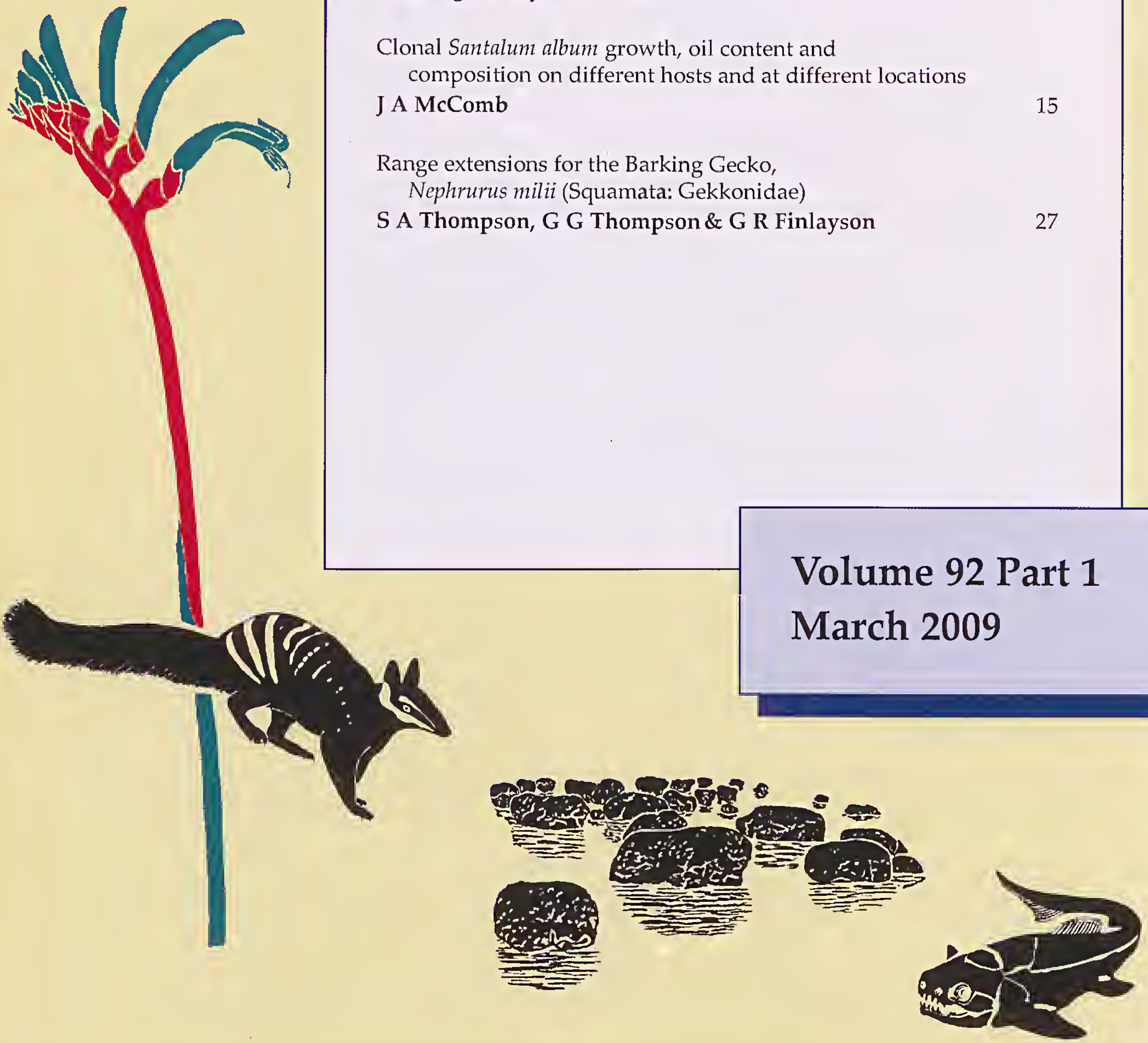

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Cover design: The four subjects symbolize the diversity of sciences embraced by the Royal Society of Western Australia. Mangles' kangaroo paw (*Anigozanthos manglesii*) and the numbat (*Myrmecobius fasciatus*) are the floral and faunal emblems of Western Australia, and stromatolites are of particular significance in Western Australian geology (artwork: Dr Jan Taylor). The Gogo Fish (*Mcnamaraspis kaprios*) is the fossil emblem of Western Australia (artwork: Danielle West after an original by John Long).

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Seedling growth responses of *Banksia littoralis* and *Melaleuca preissiana* to soil salinity

P K Groom[1] & T D Lardner[2]

[1]Centre for Ecosystem Diversity and Dynamics,
Department of Environmental and Aquatic Sciences,
Curtin University of Technology,
GPO Box U1987, Perth WA 6845.
✉ p.groom@curtin.edu.au

[2]Centre for Land Rehabilitation,
School of Earth and Environment,
Faculty of Natural and Agricultural Sciences,
The University of Western Australia, Crawley WA 6009.

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Abstract

Banksia littoralis (Swamp Banksia) and *Melaleuca preissiana* (Moonah) are two tree species that are commonly associated with wetland fringes and inter-dunal depressions of the Swan Coastal Plain. These low-lying areas are prone to hydrological disturbances and salinisation. The growth and tolerance of *B. littoralis* and *M. preissiana* seedlings to increased soil salinity was examined via a pot trial that lasted for 70 days with weekly watering of 0, 50, 100 and 150 mM of NaCl solution. No mortality was observed for either species within any of the salt treatments. *M. preissiana* seedlings showed no difference in their growth response or above-ground biomass allocation. *B. littoralis* seedlings showed a substantial reduction in relative growth rate and net assimilation rate for solutions greater than 100 mM NaCl. This resulted from an overall reduction in shoot dry mass, with less leaf area being produced per unit leaf mass, and caused recently produced leaves to become dehydrated. These results suggest that *M. preissiana* seedlings are capable tolerating soils with an electrical conductivity of at least 1650 µS cm^{-1}, whereas seedling growth of *B. littoralis* is significantly reduced for soil conductivity > 980 µS cm^{-1}. From this study it can be concluded that soil salinisation will have a more detrimental impact on *B. littoralis* seedling recruitment than co-occurring *M. preissiana* seedlings.

Keywords: Moonah, relative growth rate, salt tolerance, Swamp Banksia, Swan Coastal Plain

Introduction

Banksia littoralis R. Br. (Proteaceae) and *Melaleuca preissiana* Schauer (Myrtaceae) are two tree species from the Swan Coastal Plain which are entirely dependant on accessing groundwater for their survival, and as such are restricted to the fringes of wetlands and low-lying inter-dunal depressions that dominate the area. These low-lying areas are more prone to becoming salinised, and approximately 70% of wetlands have been lost due to increased draining, filling or land clearing since European settlement (Halse 1989). While many of the Swan Coastal Plain wetlands are not highly saline (i.e. conductivity >5 000 µS cm^{-1}), areas derived from marine, estuarine, or littoral deposits (especially some of the loamy soils surrounding wetlands) have been found to have high soluble and exchangeable sodium contents (Cochrane *et al.* 1994), dominated by sodium chloride (Halse 1989). Water quality data collected in December 1987 from 16 Perth wetlands ranged from 505 to 10 270 µS cm^{-1} (Davis *et al.* 1991), whereas more recent data collected from 19 different wetlands ranged from 0.11 to 4.51 ppt (171 to 7203 µS cm^{-1}) (John & Kemp 2006).

Hydrological alteration, nutrient enrichment and salinisation resulting from human activity are the main factors placing fringing wetland vegetation at risk (Davis & Froend 1999). For *M. preissiana,* seedling recruitment events are episodic, and over time may not compensate for the loss of older plants within populations (Froend *et al.* 1993). *M. preissiana* survives changes in the underlying hydrology by its extensive root system and moderate flood tolerance of seedlings (Froend *et al.* 1993), with any change in population structure noticeable only after many decades (Groom *et al.* 2001). The seedlings of *B. littoralis* are also flood tolerant (Groom 2004a), but are more susceptible to declining groundwater levels (Groom *et al.* 2001).

This paper compares the growth response of *B. littoralis* and *M. preissiana* seedlings to increased soil salinity. The response of these two wetland tree species to the effects of increased soil salinisation is unknown, and will have significant impacts on the species' ability to recruit in an environment already being impacted by declining groundwater levels and episodic flooding. If Swan Coastal Plain wetlands do become more saline, there are implications for the long-term survival of the fringing vegetation, with populations being unable to offset adult plant mortality by seedling recruitment.

Methods

Experimental setup

The salinity trials were conducted in a naturally lit plant house at the Bentley Campus of Curtin University of Technology. Six- to nine-month old seedlings of *B. littoralis* and *M. preissiana* were sourced from a local Perth nursery. All seedlings were grown from seeds collected from unverified natural populations inhabiting Perth's northern Swan Coastal Plain. Seedlings were transplanted into cylindrical pots (8.2 cm diameter, 14.9 cm height) about two weeks prior to the commencement of the experiment, using a potting medium composed of six parts coarse river sand, three parts composted pine bark, and one part slow release, low phosphate fertiliser (Osmocote® Native Gardens, Scotts Australia). Salinity solutions were applied weekly as analytical grade NaCl dissolved in deionised water at 50 mM, 100 mM, and 150 mM concentrations while a control received deionised water only. Watering was conducted so as to allow a small degree of flushing to occur. Five seedlings per species were randomly selected and assigned to each salinity treatment.

Harvest and growth parameters

Four seedlings per species were harvested the day before the salinity trial commenced. Shoots were excised from roots and divided into leaf and stem components for each seedling. After 70 days of watering with NaCl solutions all surviving seedlings were similarly harvested and partitioned into their stem, leaf and root components. In addition, the fresh weight of the youngest fully expanded leaf (YFEL) from each seedling was measured. Fresh leaves of each plant were digitally scanned and then analysed for projected area using the Image J software (http://rsb.info.hih.gov/ij). All plant material was then oven dried at 70°C for 72 hours, after which dry weights of all components were taken.

Relative growth rate (RGR) and net assimilation rate (NAR) was calculated according to McGraw & Garbutt (1990). In addition, the leaf area ratio (LAR), measured as the ratio of projected leaf area (photosynthetic surface) to aboveground biomass; leaf weight ratio (LWR), calculated as a percentage biomass allocation to leaves; specific leaf area (SLA), measured as total leaf area divided by total leaf mass were calculated. For the youngest fully expanded leaf of each seedling, the degree of succulence (measured as leaf water content per unit leaf area) and SLA were determined.

Soil analysis

Soil samples were collected for each species-treatment combination after 70 days watering with their respective salt solution, and allowed to air dry for two weeks. Subsamples were then mixed at a rate of 1:5 with deionised water, shaken for one minute, and allowed to stand for approximately five minutes. Electrical conductivity was then recorded using a portable meter (WP-81, TPS, Brisbane, Australia) from the supernatant.

Statistical analyses

Interaction between species and treatment blocs was analysed by factorial ANOVA and statistical difference between treatments within species groups was analysed by one-way ANOVA using SPSS version 15.0 (SPSS Inc. Chicago, USA). Homogeneity of variances was assessed using Levene's test and log or root transformed as required, with data presented as untransformed means. Scheffé's test was used for multiple comparisons of means at a significance level of 0.05.

Results

There was no mortality recorded for either species in any of the salt treatments. *Banksia littoralis* showed a general reduction in shoot biomass with increasing salinity. Seedlings growing in 150 mM NaCl had a 37% reduction in biomass compared to the controls (Table 1), resulting in the lowest relative growth rate and net assimilation rates (a measure of overall efficiency of a seedling in generating biomass) recorded. The addition of different levels of salt solutions had no significant impact on leaf area ratio (a measure of leafiness) or leaf weight ratio (a measure of biomass allocation to leaves) but did result in a significant decrease in specific leaf area (a measure of relative leaf density) at 150 mM NaCl. Salinity levels greater than 100 mM NaCl resulted in less leaf area being produced per unit leaf weight, and less water occurring in the youngest fully expanded leaves per gram dry weight (Table 1).

In contrast to the *Banksia*, *M. preissiana* displayed no appreciable change in shoot biomass across treatments (Table 2), with no change in relative growth rate or net assimilation rate. There was no significant difference in seedling leaf area ratio, specific leaf area or degree of succulence. Overall *M. preissiana* seedlings grew faster, with a higher leaf area ratio and specific leaf area than *B. littoralis*.

After 70 days of watering with salt solutions the soil electrical conductivity increased as the concentration of salt solution increased (Table 3), with the conductivity data for *M. preissiana* soil being 244 to 560 µS cm^{-1} higher than for *B. littoralis* soil at the two highest NaCl concentrations.

Discussion

It can be expected that species restricted to winter-wet depressions, wetlands and boarding water courses in southwestern Australia should display some degree of salt tolerance. On the Swan Coastal Plain, vegetation occupying low-lying inter-dunal areas or fringing wetlands are the most prone to environmental change. Our data suggests that *M. preissiana* seedlings are capable of growing in soils with an electrical conductivity of 1650 µS cm^{-1} for at least 70 days without any adverse effect on seedling growth or biomass allocation. This is higher than the known salt tolerance limits for *M. preissiana* trees (500–1000 µS cm^{-1}; Bennett & George 2002). In contrast, it is expected that *B. littoralis* seedlings would not tolerate prolonged exposure to soil electrical conductivities greater than 1090 µS cm^{-1} due to a decline in relative growth and net assimilation rates. At soil conductivity greater than 980 µS cm^{-1} *B. littoralis* seedlings respond to the increased salt by a reduction in shoot weight and total leaf area produced, resulting in a significant

Table 1

Growth parameters of *Banksia litoralis* seedlings after 70 days of watering with different concentrations of NaCl solution. Data presented as means (se) for 5 seedlings. Superscripts indicate subsets between treatments based on Scheffé's test after differences were found by one-way ANOVA. % change is in relation to control (0 mM) data. SLA = specific leaf area; YFEL = youngest fully expanded leaf; RGR = relative growth rate; NAR = net assimilation rate. ns = not significant ($P > 0.05$)

					P value	% change		
	0 mM	50 mM	100 mM	150 mM		50 mM	100 mM	150 mM
Shoot weight (g)	1.8^{a} (0.1)	1.8^{a} (0.2)	1.4^{ab}(0.1)	1.2^{b} (0.04)	0.001	–1	–23	–37
Leaf area ratio (cm^2 g^{-1} shoot)	51.7 (3.8)	52.1 (2.7)	49.2 (1.9)	49.8 (1.6)	ns	1	–5	–4
Leaf weight ratio (% leaf shoot^{-1})	67.4 (3.3)	66.5 (2.9)	68.6 (2.8)	72.9 (1.4)	ns	–1	2	8
SLA (cm^2 g^{-1})	76.6^{a} (3.3)	78.3^{a} (1.6)	71.8^{ab} (1.3)	67.8^{b} (1.5)	0.006	2	–6	–11
SLA-YFEL (cm^2 g^{-1})	82.5^{a} (7.1)	87.7^{a} (7.2)	68.4^{b} (1.8)	68.6^{b} (3.0)	0.030	6	–17	–17
Succulence-YFEL (mg g^{-1})	20.8^{a} (2.7)	19.4^{a} (0.6)	14.0^{b} (2.0)	14.3^{b} (1.7)	0.040	–6	–32	–31
Shoot RGR (mg g^{-1} d^{-1})	6.7	6.6	3.1	0.1	–	–2	–55	–98
Shoot NAR (mg g^{-1} d^{-1})	0.130	0.127	0.062	0.002	–	–3	–52	–98

Table 2

Growth parameters of *Melaleuca preissiana* seedlings after 70 days of watering with different concentrations of NaCl solution. Data presented as means (se) for 5 seedlings. See Table 1 for description of abbreviations.

					P value	% change		
	0 mM	50 mM	100 mM	150 mM		50 mM	100 mM	150 mM
Shoot weight (g)	2.2 (0.1)	1.9 (0.2)	2.3 (0.3)	2.2 (0.2)	ns	–12	6	–7
Leaf area ratio (cm^2 g^{-1} shoot)	90.1 (3.5)	101.8 (9.8)	87.9 (8.7)	93.5 (1.8)	ns	13	–2	4
Leaf weight ratio (% leaf shoot^{-1})	73.2 (5.8)	74.5 (1.6)	74.4 (2.3)	77.2 (0.1)	ns	2	2	5
SLA (cm^2 g^{-1})	126 (12)	136 (12)	117 (8.4)	121 (2)	ns	9	–7	–4
SLA-YFEL (cm^2 g^{-1})	114 (7)	132 (7)	123 (20)	126 (4)	ns	16	8	10
Succulence-YFEL (mg H_2O g^{-1})	11.0 (0.4)	13.6 (1.3)	11.3 (1.5)	12.7 (0.8)	ns	24	3	16
Shoot RGR (mg g^{-1} d^{-1})	21.3	19.4	22.1	20.3	–	–9	4	–5
Shoot NAR (mg g^{-1} d^{-1})	0.236	0.191	0.251	0.217	–	–19	6	–8

Table 3

Soil electrical conductivity readings (µS cm^{-1}) after 70 days of watering with salt solutions. Data are means of 2 replicates.

	0 mM	50 mM	100 mM	150 mM
B. littoralis	210	933	984	1090
M. preissiana	114	986	1228	1650

decrease in specific leaf area (a measure of relative leafiness), as well as significant dehydration of the youngest fully expanded leaf.

Soil salinity levels have important implications on the recruitment, growth and survival of the native vegetation fringing wetlands, particularly when salinity levels rise beyond naturally occurring levels due to human interference. Seedling establishment is crucial for the long-term viability of *B. littoralis* and *M. preissiana* populations, with recruitment events only occurring episodically when conditions are suitable. For *M. preissiana*, recruitment tends to occur on mass during the winter months following a fire, when there is sufficient soil moisture to ensure adequate root growth (Froend *et al.* 1993). Seedling recruitment within the fringing embankments is influenced by wetland water levels (Froend *et al.* 1993) with seedling long-term survival ultimately dependent on underlying groundwater levels. For both species, an increase in soil salinity would have a detrimental impact on seedling recruitment levels, although clearly *M. preissiana* is more salt tolerant than *B. littoralis*.

The decline in *B. littoralis* and *M. preissiana* population vigour on Perth's Swan Coastal Plain over the past 40 years has been attributed to a regional decline in groundwater levels, resulting from the combination of declining annual rainfall and localised groundwater abstraction (Groom *et al.* 2001). Deep-rooted species fringing wetlands are more prone to water stressors because they are obligately dependent on subsurface groundwater (Groom 2004b; Canham *et al.* 2009). *B. littoralis* and *M. preissiana* seedlings can survive flooding conditions for at least one month (Froend *et al.* 1993; Groom 2004a) but are unable to survive periods of submergence. Studies on species salt tolerance typically investigate interaction with waterlogging, as together these stressors can cause severe damage to plants (Barrett-Lennard 2003). Although the combined effect of salinity and waterlogging on the growth and physiology of our two target species is unknown, it has been reported that waterlogging with freshwater has little effect on the growth and survival within the genus (van der Moezel *et al.* 1991). For *Melaleuca* species inhabiting areas prone to inundation, experimental pot trials have shown a tolerance of at least 42000 $\mu S\ cm^{-1}$ soil conductivity with 22 days of waterlogging (*M. cuticularis*; Carter *et al.* 2006) and 62000 $\mu S\ cm^{-1}$ after waterlogging for 10 weeks (*M. ericifolia*; Salter *et al.* 2007). Soil salinisation is well known to inhibit growth, alter plant morphology and leaf physiology in species not adapted to tolerate salinity (Kozlowski 1997). If Swan Coastal Plain wetlands become more saline the survival and population vigour of species restricted to wetland fringes and low-lying depressions will be negatively impacted.

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Observations of the reproductive biology of *Acacia saligna* (Labill.) H.L. Wendl.

N George[1,3]*, M Byrne[2,3] & G Yan[1]

[1]School of Plant Biology,
Faculty of Natural and Agricultural Sciences,
The University of Western Australia,
35 Stirling Highway, Crawley, WA 6009, Australia.
[2]Science Division, Department of Environment and Conservation,
Locked Bag 104, Bentley Delivery Centre, WA 6983, Australia.
[3]Co-operative Research Centre for Plant-based Management of Dryland Salinity,
The University of Western Australia,
35 Stirling Highway, Crawley, WA 6009, Australia.
*Corresponding Author: Present address: Department of Horticultural Science,
North Carolina State University.
✉ Nicholas_George@ncsu.edu

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Abstract

Acacia saligna (Labill.) H.L. Wendl. (subgenus Phyllodineae) has the potential to be developed as a biomass crop for the agricultural regions of southern Australia. This study examined the reproductive biology of *A. saligna* to facilitate the domestication and utilization of the species. The reproductive biology of *A. saligna* was found to be broadly similar to other Australia Acacia in the subgenus Phyllodineae. Flowering conformed to the phases described for other Acacia species. The flowers of *A. saligna* open asynchronously within a flower-head. Female sterility also was observed to occur in *A. saligna* with extent of sterility being highly variable. Mean reproductive output was found to be low. This work has identified a number of characters that will potentially make controlled crossing in *A. saligna* practically challenging. If a breeding program for the species is to be established then using open pollination techniques may be more practical and cost effective than controlled crossing.

Keywords: *Acacia saligna*, reproductive biology, floral biology, pollen quality, pollen storage and reproductive output.

Introduction

Acacia is a genus comprising more than 1,500 species, with the greatest diversity of species being native to Australia (Maslin *et al.* 2003). The majority of these species can be referred to the subgenus Phyllodineae (Maslin *et al.* 2003). Despite the large number of species in the genus the reproductive biology of only a limited number of species have been examined in detail (Kenrick 2003). The general characteristics of reproductive biology tend to be similar in all species of Acacia studied so far, though subtle differences between species have been identified (Kenrick 2003; Kenrick & Knox 1989; Sedgley 1989). Slight differences in reproductive biology can impact on utilization and management (Sedgley & Griffin 1989; Simmonds & Smartt 1999), so it will be necessary to examine the reproductive biology of all Acacia species of interest for utilisation in restoration and agroforestry.

A study that assessed the woody-crop potential of 462 Acacia native to the dryland cropping regions of southern Australia identified 35 species with good potential for domestication (Maslin & McDonald 2004). *Acacia saligna* (Labill.) H.L. Wendl. was ranked as one of the best of the 35 species because of its fast to very fast growth rates, ability to produce moderate to large amounts of biomass, coppicing potential in some genotypes, moderate to vigorous suckering, and potential to be cultivated over a reasonably wide geographic area. Acacia saligna is native to the south-west of Western Australia and is already planted extensively around the world.

Economically important traits, such as plant form, are known to be variable in *A. saligna* (Le Houerou 2002; Le Houerou & Pontanier 1987; Maslin *et al.* 1998; Nativ *et al.* 1999), and some of the variation may be the result of genetic diversity in the species, which suggests that the cultivation of improved cultivars could be possible (George *et al.* 2006). Development of improved cultivars via selection and breeding will enhance the value of the species as a crop plant. Breeding and cultivation of *A. saligna* will require information regarding the reproductive biology of the species (Sedgley 1996). This paper presents findings from an examination of the reproductive biology of *A. saligna* that will provide information useful for the both the domestication of the species.

Materials and methods

Acacia saligna exhibits considerable morphological variation. Four informal morphologically differentiated variants are currently recognized and at the time of writing the intention is to raise these to subspecies rank (Maslin in prep.). The subspecies are presently designated as *lindleyi*, *pruinescens*, *saligna* and *stolonifera*.

Study sites

A range of study sites were used in this research. The sites are listed in Table 1, together with latitude and longitude co-ordinates, information on subspecies present, and year sampled. The subspecies occupy separate but overlapping distributions in south-western Australia (Figure 1).

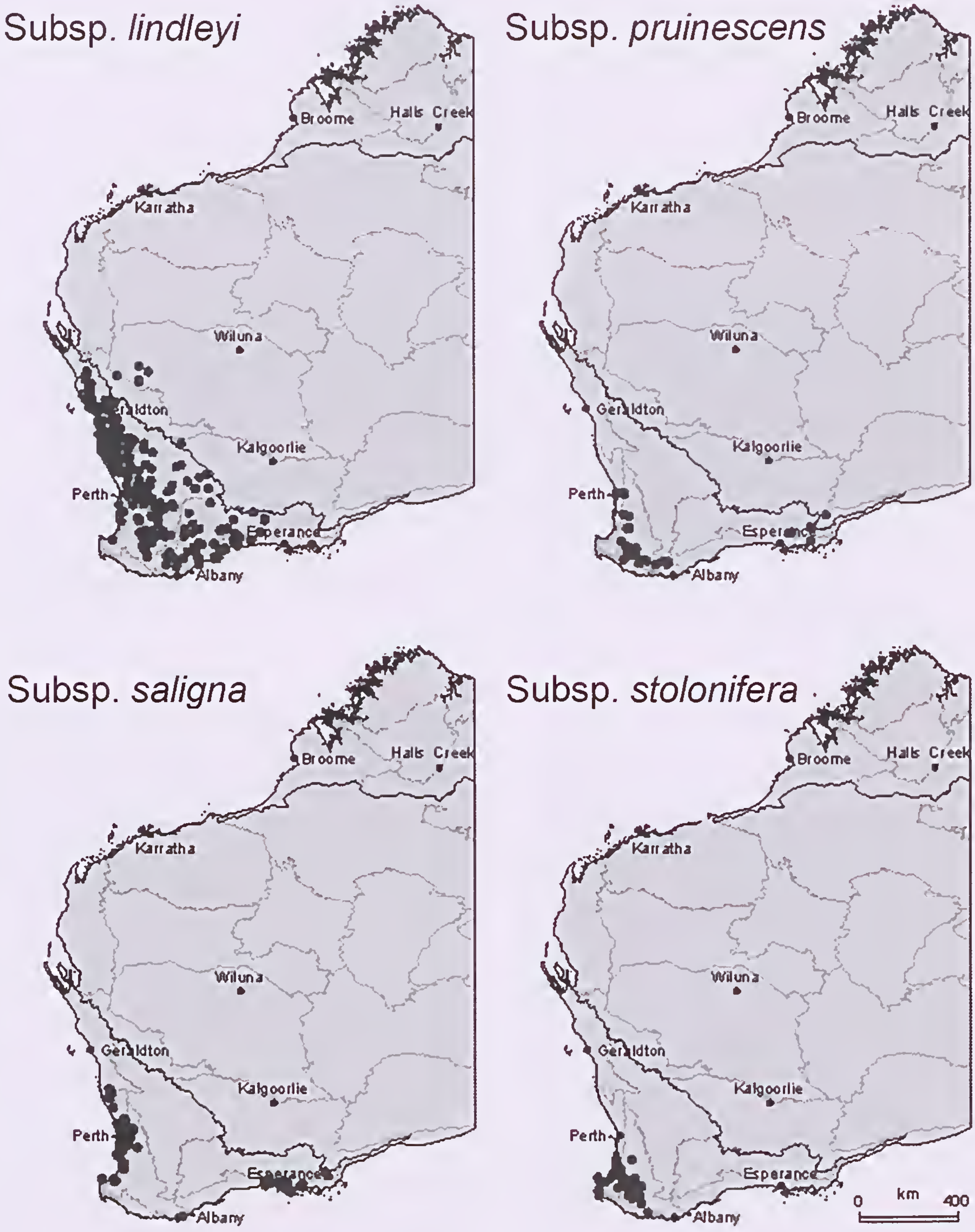

Figure 1. The ranges of the subspecies of *Acacia saligna* in south-western Australia (WAH 2009).

Table 1

Details of the sites used to assess reproductive output in *Acacia saligna*.

subspecies	Population Name	Year sampled	Lat. E	Long. S	WAHERB Sheet no.
saligna	Flynn	2005	31 41 14	115 46 03	6796273
saligna	Yunderup	2003/2005	32 34 20	115 47 27	6451411
saligna	Millar	2005	32 17 09	115 47 45	7285752
stolonifera	Hesters	2003/2005	33 54 19	116 06 22	6020232
stolonifera	Peppermint Gr	2003/2005	33 32 24	115 30 30	6020275
stolonifera	Leschenault	2003	33 13 04	115 41 36	6451438
stolonifera	Lowden	2005	33 32 32	115 56 51	7253176
pruinescens	Tweed Road	2003/2005	34 00 06	116 13 08	6020208
pruinescens	Tweed River	2005	34 00 21	116 29 31	6183336
pruinescens	Weinup	2005	34 15 29	116 271 5	7255144
lindleyi	Wickepin	2003/2005	32 37 48	117 23 01	6183174
lindleyi	Jilakin	2003	32 39 50	118 19 45	6183190
lindleyi	Moore River	2003/2005	31 03 54	115 33 10	6020283
lindleyi	Towerninning	2005	33 35 13	116 47 49	7252552

WAHERB Western Australian Herbarium

Floral biology

Aspects of floral biology examined in this study included temporal changes occurring during floral development (over daily and hourly timescales), the timing of stigmatic receptivity, and female sterility.

The progression of floral development was observed on trees of *A. saligna* subsp. *saligna*. Racemes with unopened flowers were tagged on six trees growing in the Matilda Bay Reserve. Individual flower heads within the racemes were observed every day at 10 a.m. for 12 days (flower head is used to refer to the globose collection of individual flowers). Attention was paid to the relative timing of the emergence of the style and stamens, pollen shed and the duration of individual flower heads.

Flowering events occurring on shorter time-scales were examined using the methods described by Sornsathapornkul & Owens (1998) and Tandon & Shivanna (2001). Whole branches were taken from six trees at the same reserve and 32 unopened but mature flower heads were collected from each branch, with 3 to 4 mm of peduncle left attached to each flower head. The flower heads were washed in distilled water to remove pollen, insects and other debris. Flower heads were then placed in Petri dishes containing agar (0.8% agar, 1% sucrose) by inserting the peduncle into the agar until the base of the flower head touched the agar media. The flower heads from a single tree were divided between two dishes with 16 heads per dish. The Petri dishes, with lids, were placed on a laboratory bench under fluorescent lighting. The temperature of the room was maintained at approximately 20°C throughout the experiment. Individual flower heads were observed via dissecting microscope every two hours for 32 hours. The number of flowers open within flower heads, the timing of the emergence of styles and stamens and the length of time taken for floral development were recorded.

The timing of stigmatic receptivity was determined using both enzymatic activity of stigmas and pollen tube growth. To assess enzymatic activity of stigmas, ten inflorescences were tagged on each of four trees of the subspecies saligna in the Matilda Bay Reserve. Flower heads were harvested at 10 a.m. at 0, 1, 2, 3 and 4 days after the first flowers in each flower head opened. The flower heads were immersed in 0.5 mg/ml α-naphthyl acetate in acetone, 0.1 M phosphate buffer, 2.5 mg/ml Fast Blue B Salt for 5 to 10 minutes. This stain indicates non-specific esterase activity, which is an indicator of stigmatic receptivity (Kenrick & Knox 1981). Mature and immature flower heads in which no flowers had opened were also sampled, dissected and stained.

To assess pollen tube growth, individual flowers from 16 mature flower heads were harvested from the receptacle as they opened, placed in agar plates and observed every 2 hours. Individual flowers were hand pollinated at 2, 10, 20 and 30 hours following anthesis. Before and after pollination the individual flowers were supported in agar plates (0.8% agar, 1% sucrose). Fresh pollen for pollination was collected by beating newly opened flowers on a clean microscope slide. The risk of selfing adversely affecting the results of this study was considered to be low because studies have shown no difference in pollen tube growth between selfing and outcrossing in *Acacia* (Kenrick *et al.* 1984). Despite this, to reduce the risk of genetically similar individuals affecting the results, trees used as a source of pollen were located approximately 1 km from the study population. To perform the cross, a single polyad was placed directly on the stigma of each flower under a dissecting microscope. Following pollination flowers were left for 12 hours and then fixed in glacial acetic acid:ethanol (1:2) at 5°C for 24 hours. To observe pollen tube growth in open pollinated flowers mature flower heads of *A. saligna* subsp. *saligna* were also harvested from the Matilda Bay Reserve populations and fixed.

A modified method of Kenrick & Knox (1985) was used to visualize pollen tubes. Flowers were soaked overnight in 8M NaOH and then stained using aniline blue (0.1% aniline, pH 4.5). Flowers were rinsed well in distilled water between each step. The prepared slides were kept at 5°C for 2 hours prior to viewing pollen tubes using fluorescent microscopy. Pollen tubes were ranked on either their presence or absence and a qualitative assessment was made regarding the number of tubes per style and their length.

The presence of female-sterility was examined in eight wild populations of *A. saligna* in 2003. The populations examined were Yunderup (ssp. *saligna*); Hesters, Peppermint Gr. and Leschenault (ssp. *stolonifera*); Tweed Road (ssp. *pruinescens*); and Wickepin, Jilakin and Moore River (ssp. *lindleyi*) (Table 1). Three trees were sampled in each population. Six flower heads from each tree were dissected and examined under dissecting microscope. A flower was considered female-sterile if the gynoecium was absent or visibly malformed.

Pollen viability and pollen storage

To test the efficacy of different storage treatments in maintaining the viability of *A. saligna* pollen, flower heads were harvested from three trees at the Matilda Bay Reserve population, with 200 flower heads harvested per tree. Inflorescences were spread evenly on paper and left to air dry at 20°C for 4 hours before being bulked. Fine scissors were used to cut anthers from the inflorescence filaments to reduce the volume of material to be desiccated. The material from each tree was bulked and dried for 48 hours at either a) 5°C in a desiccator over a large volume of silica or b) in a HetoFD40 freeze drier at $1 \times 10^{-1.6}$ hPA. To prevent condensation following desiccation dried material was left in a desiccator and placed on the bench to warm to room temperature.

Samples of anthers were taken from the bulked material and put in Eppendorf tubes that were stored in zip-lock bags with ~30 g of silica. Bags from each treatment were then randomly selected and stored at either room temperature, 5°C, –20°C or –80°C. The viability of the pollen was tested at 9, 30, 59, 163 and 251 days. The viability of the pollen was assessed using the methods of Dafni (1992) and Sedgley and Harbard (1993). Flowers were placed in a 20% sucrose solution and agitated to release polyads. The suspension of polyads was left for 10 minutes to allow the grains to hydrate. A drop of 1% Fluorescein Diacetate (FDA) in acetone was evaporated on a microscope slide and a drop of the polyad-suspension added. The stained polyads were then viewed via fluorescence microscopy. Fluorescing grains were regarded as being viable. At least 50 polyads were scored for each sample and the relative numbers of viable polyads recorded. Due to the compound nature of *Acacia* pollen, viability was scored according to the method of Kenrick & Knox (1989) – if more that 50% of the grains within the polyad were fluorescing the grains was scored as being greater than fifty percent viable, and if less than 50% but more than 0% were fluorescing it was scored as being less than fifty percent viable. A score of zero viability was also applied if no grains were fluorescing.

Pod set

The pod set of *A. saligna* was assessed using similar methods to those described by Yates & Broadhurst (2002) and Gaol & Fox (2002). Examinations were carried out in the flowering seasons of 2003 and 2005. At each of the 14 populations listed in Table 1, 15 mature trees spaced at approximately 10 metre intervals were tagged. On each tree three 40 cm lengths of branch (measured from the apical tip and including lateral branches) were tagged. The branches were spaced throughout the flowering canopy. The number of open and unopened flower heads distal to each tag were counted. After pods had developed, but not fully matured and dehisced, the branchlets were collected and taken back to the laboratory and pod production was recorded.

Statistical analysis

All statistical analysis was carried out using the programs Genstat (Genstat 2002) and SPSS for Windows (SPSS 2008). Tests for significant differences between groups of normally distributed data were carried out using ANOVA and pairwise comparisons with Tukey's Least Significant Difference (Genstat 2002). Data regarding female sterility and reproductive output was found to be non-normally distributed. The closest approximation for these data sets is an exponential distribution so a statistical method for comparing exponential distributions was used to test for significance between the subspecies means (Weerahandi 2003).

Results

Floral biology

In wild trees, individual flowers in a flower head were observed to open asynchronously, and flower heads within inflorescences were also observed to open asynchronously, usually with basal flower heads opening first. Opening of flowers within an inflorescence occurred over a period of 1 to 2 days. Once all the flowers were open, the flower head persisted for 5 to 7 days until the individual flowers began to detach from the receptacle. The pistil and stamen appeared to emerge either synchronously or in quick succession. When the flowers in a flower head were fully open, the style was typically around 1 mm longer than the stamens. Pollen release occurred over a period of 1 to 2 days after the flowers opened.

Observations of floral opening in the laboratory showed the first event was splitting of the perianth in individual flowers (Figure 2A and B) which was then followed by the emergence of a 'tuft' of anthers 1 to 2 mm in length (Figure 3A). Following the emergence of the anther-tuft the style elongated, followed shortly by elongation of stamens (Figure 3B and C). The non-elongated style was folded inside the flower bud (Figure 4). The opening of the flowers in a flower head was asynchronous, the basal flowers were typically the first to open. Throughout the process of floral opening the style typically remained 1 to 2 mm longer than the stamens (Figure 5A and B). In 12% of observations the fully extended stamens elongated to a length comparable with the styles.

The time period between perianth splitting and emergence of the anther-tuft was between 2 and 14 hours, but typically less than 8 hours (Figure 6A). The style emerged before the stamens elongated in 59% of cases, whereas both the style and stamens emerged together in 41% of cases. When the style emerged alone, it reached a length of 1 to 2 mm before the stamens began to elongate, which was typically 2 to 8 hours later (Figure 6B). The remaining flowers in a head took an average of

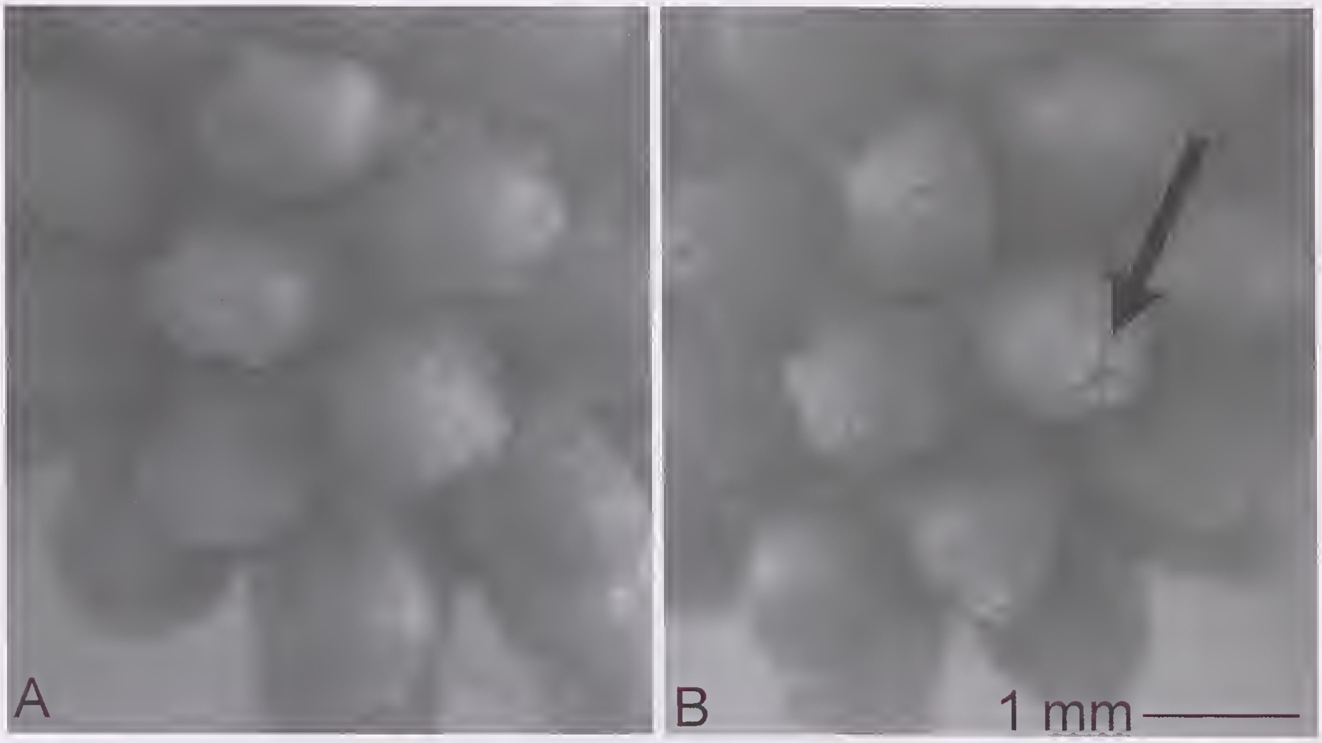

Figure 2. An inflorescence of *Acacia saligna* showing (A) unsplit perianth and (B) split perianth (arrowed).

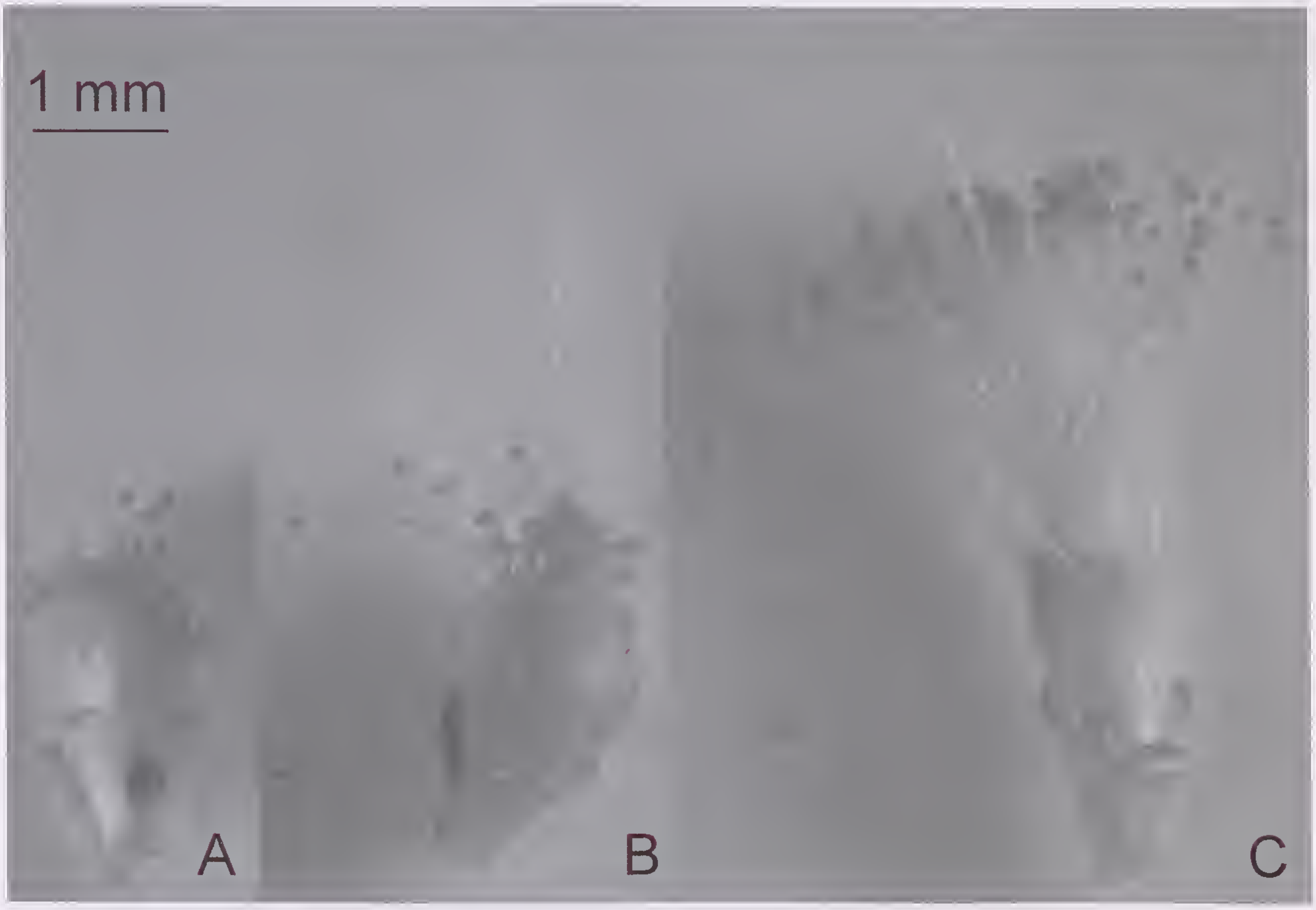

Figure 3. Individual flowers of *Acacia saligna* showing sequentially the (A) newly opened flower with stamen 'tuft', (B) elongation of style, and (C) elongation of stamens.

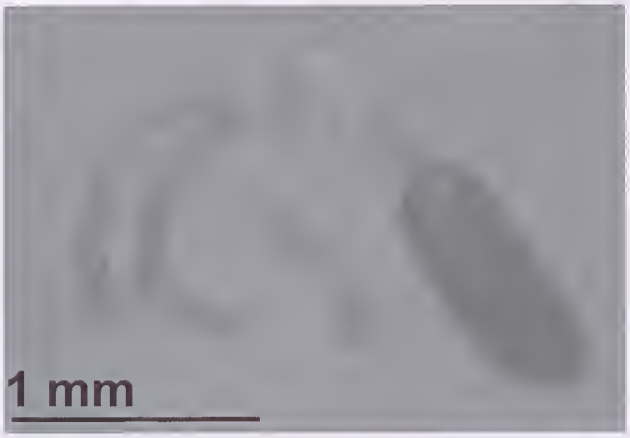

Figure 4. A gynoecium from unopened flower, showing folding of style.

9 hours to open, with a standard deviation of 5 hours (Figure 6C).

Drops of fluid were observed on the stigma of some flowers between 10 and 26 hours following opening. In addition, no pollen shed was observed in any of the flowers in the laboratory. The samples were in closed Petri dishes so it was suspected that failure to shed pollen due to humidity. Upon completion of the laboratory observations the tops were removed from the Petri dishes and as the flowers were allowed to air-dry pollen-shed was observed.

Enzymatic activity, as indicated by staining, was not observed in stigma from immature buds (Figure 7A). Stigma from mature unopened-buds, and flowers open for 1, 2, 3, and 4 days, exhibited enzyme activity (Figure 7B and C). Some stigma with pollen grains present failed to show enzymatic activity (Figure 7D). Only a single pollen grain was observed on each stigma (Figure 7C and 7D).

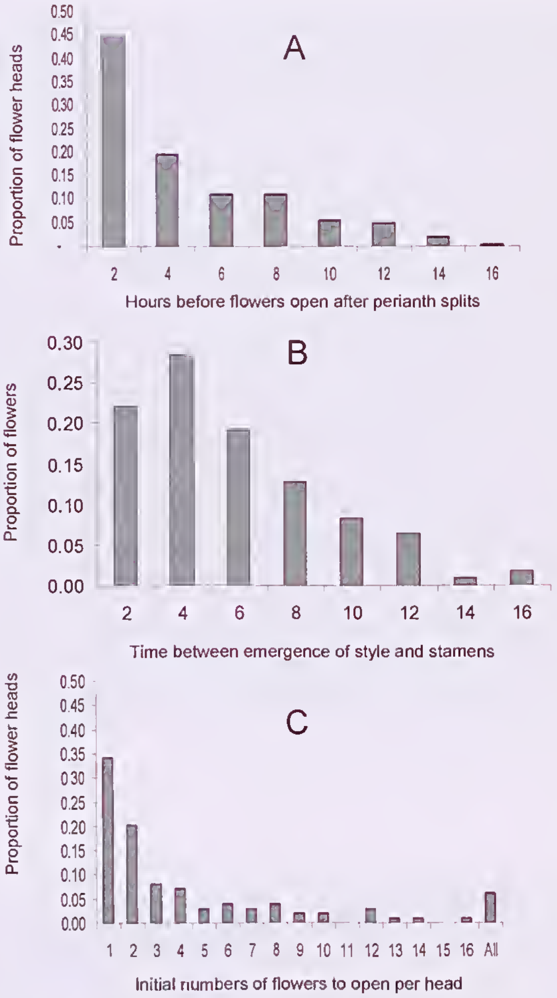

Figure 6. Observations of the time of the opening of flowers of *Acacia saligna*. (A) Time between the splitting of the perianth and the emergence of stamens, (B) Time (hrs) between the emergence of the style and the emergence of stamens. (C) The initial number of flowers to open in each head.

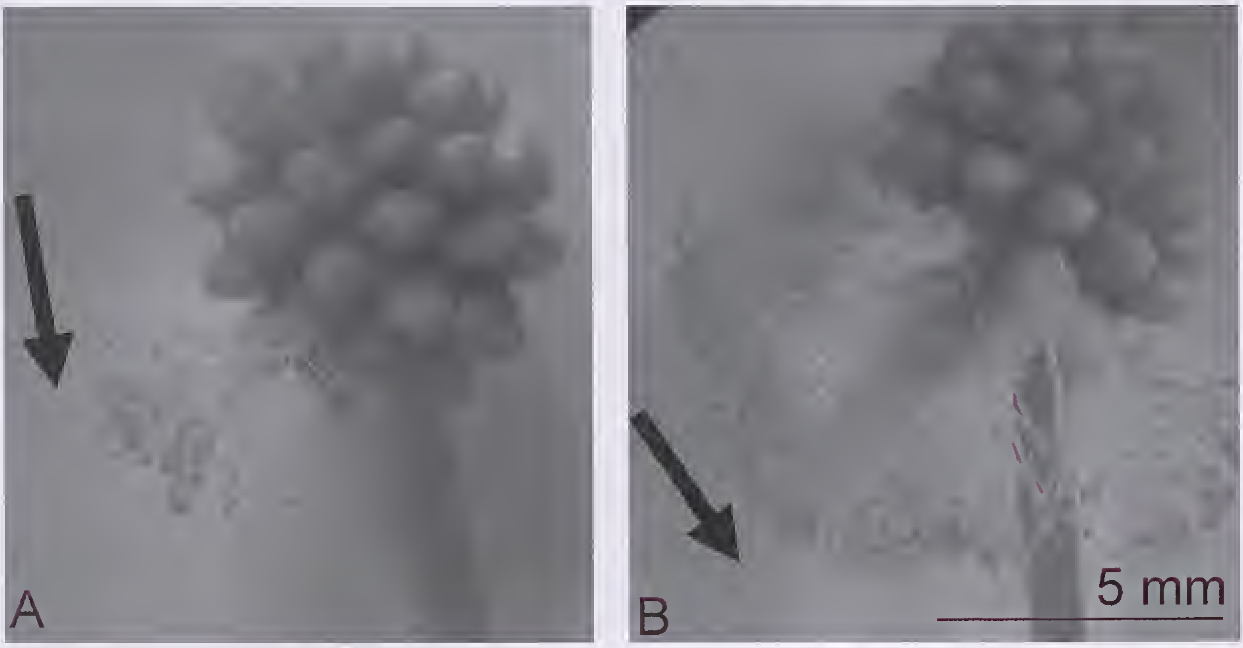

Figure 5. Separate inflorescences of *Acacia saligna* showing asynchronous opening of individual flowers and the differing lengths of styles and stamens (arrowed).

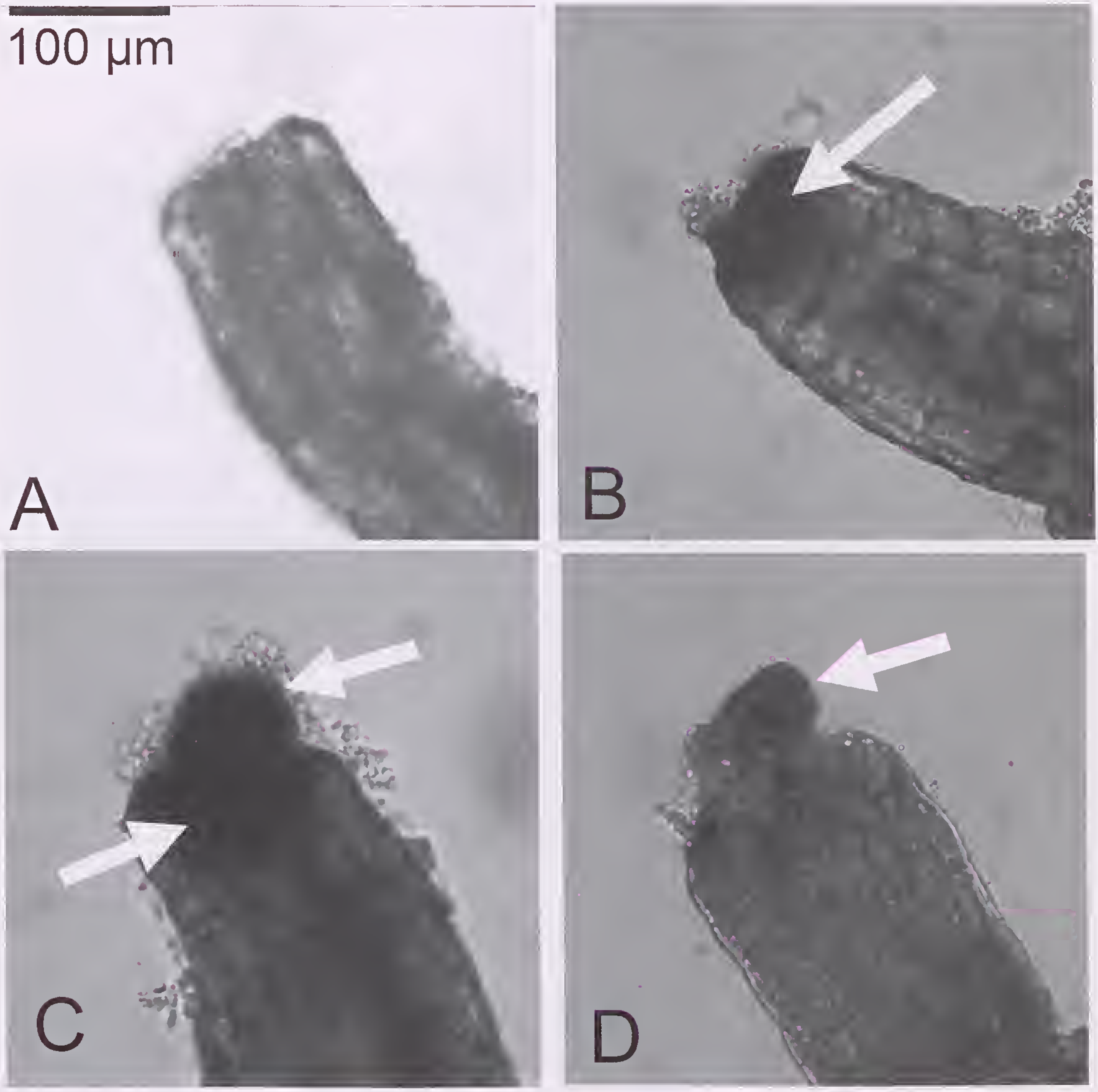

Figure 7. Stigma of *Acacia saligna* stained using á-naphthyl acetate and fast blue B salt to indicate esterase activity. Esterase activity indicates potential stigmatic receptivity. (A) A stigma from an immature bud showing no enzyme activity. (B) A stigma from a mature unopened flower showing enzyme activity (arrowed), the stigma of mature but unopened flowers show similar activity. (C) Stigma with pollen grain (arrowed). (D) Stigma with pollen grain showing no staining reaction.

Pollen tube growth was observed in flowers pollinated by hand in the laboratory. Pollen tubes were observed in the styles of flowers pollinated 2 and 10 hours after anthesis, although none of the tubes had reached the ovules. Pollen tubes were also observed in the styles of flowers pollinated 20 and 30 hours after anthesis. Some pollen tubes had reached the ovules in these flowers. Exudate was present on the stigmas of 10, 20 and 30 hour old flowers prior to hand pollination, and was inevitably blotted off when the polyads were applied. Exudate was seen to return following pollination. This pre-pollination exudate was not observed on 2 hour old flowers but exudate was observed following pollination in these flowers.

Female sterility was present in all subspecies of *A. saligna* as reduced gynoecia that were orange in colour (Figure 8A and B). The character was highly variable, with the mean value for female sterility across all populations being 10 % with a standard deviation of 17 %. The maximum number of female-sterile flowers in a flower head was 96%. There was no significant difference in the occurrence of female sterility between populations or between subspecies.

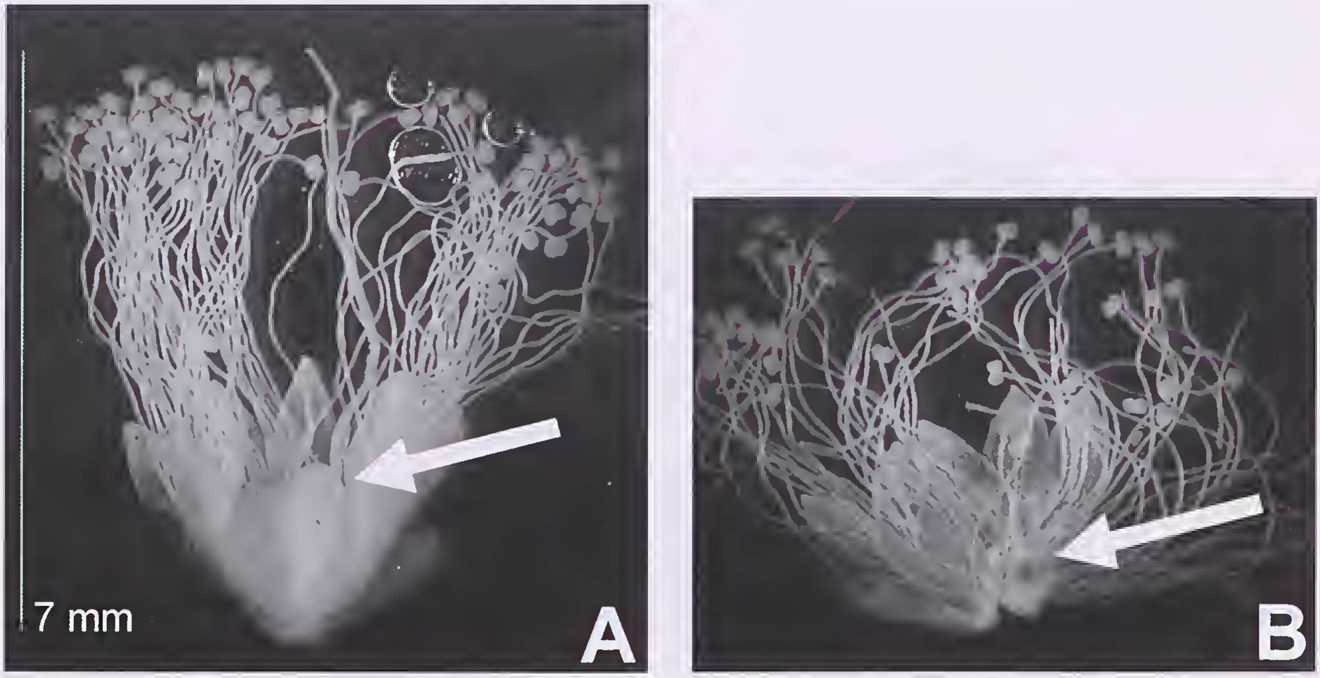

Figure 8. Individual flowers of *Acacia saligna* showing (A) a hermaphroditic flower with a normal gynoecium (arrowed) and (B) a female sterile flower, with reduced gynoecium (arrowed).

Pollen viability and pollen storage

Initial tests showed that pollen stored without being desiccated quickly lost viability. The viability of undesiccated pollen decreased to 0% after eight days stored at room temperature. When kept at –20°C 4% of the polyads retained >50% viability after 33 days, but this had decreased to 0% after 61 days. When kept at –80°C 2% of the polyads had retained >50% after 153 days. Based on these findings a non-desiccated treatment was not pursued in further experiments.

The viability of desiccated pollen was found to decrease to 0% after 163 days when stored at room temperature but retained some viability after 251 days when stored at 5°C, –20°C and –80°C (Figure 9). After 251 days storage, the average number of polyads with >50%viablity was between 0–5%. The number of polyads with <50% viability was also low but there were significant differences between storage temperatures and desiccation methods. The samples desiccated at 5°C using silica gel and then stored at low temperatures had significantly higher viability than those stored at room temperature ($p<0.01$)). The mean viability of pollen desiccated using a vacuum was lower than the pollen desiccated using silica however the difference was not significant.

Pod set

The pod set of *A. saligna* is summarized in Table 2. The pod set was found to differ significantly between the subspecies. The ssp. *pruinescens* and *saligna* had significantly higher pod set than the other subspecies and did not differ significantly from each other, whilst ssp. *stolonifera* and *lindleyi* both differed significantly from each other.

Table 2

A summary of the pod set of *Acacia saligna*. Commonality of letters indicates non-significant difference between means at the 5% level. Standard deviation in parentheses.

Subspecies	Pod set
Overall	$0.08^{(0.13)}$
lindleyi	$0.05^{(0.10)a}$
stolonifera	$0.08^{(0.14)b}$
saligna	$0.11^{(0.14)c}$
pruinescens	$0.12^{(0.15)c}$

Discussion

The reproductive biology of the various subspecies of *A. saligna* was found to be broadly similar to other Australian *Acacia* species. The flowering phases observed in ssp. *saligna* generally conform with the phases of flowering described for other *Acacia* species by Knox *et al.* (1989), being protogynous, with the style emerging before the stamens. The apparent concurrent emergence of the style and stamens in some instances may be an artefact of the 2 hour time period between observations.

The stigma of ssp. *saligna* were found to be receptive to pollen upon emergence, as has been observed in other *Acacia* (Kenrick 2003). This may represent a brief female-phase which lasts a few hours prior to the elongation of the stamens. Generally though, the female and male phases appear to overlap, with the stigma remaining receptive to pollen for at least 30 hours after emerging, while all the stamens had elongated and begun to shed pollen.

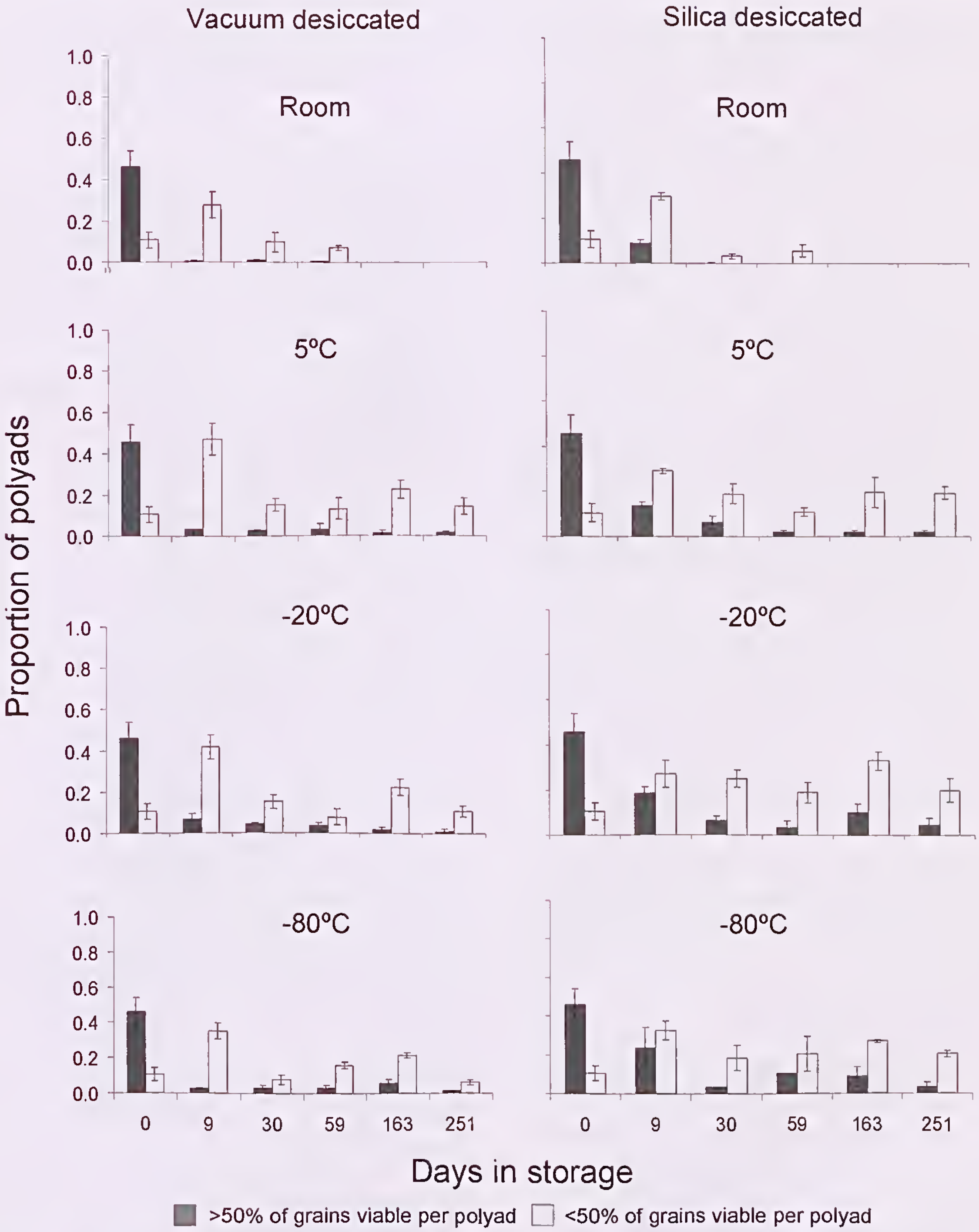

Figure 9. The viability of pollen of *Acacia saligna* following storage at different temperatures. Standard deviation is indicated.

The timing of the male phase is unclear because pollen shed appears to be mediated by ambient humidity levels. This has also been observed in other *Acacia* (Stone *et al.* 2003; Stone *et al.* 1998). Some pollen shed may be possible only a few hours following the emergence of stamens provided conditions are dry, however peak pollen shed appears to occur 24 hours after the stamens emerge.

The length of the female and male phase, and duration of the flowers, in ssp. *saligna* is within the range seen in other Australian Acacia where the female phase ranges from 1 to 10 days in length, male phase from 1 to 8 days, and flower head longevity varies between 4 and 12 days (Stone *et al.* 2003; Stone *et al.* 1998).

Acacia species within Phyllodineae show both synchronicity and asynchronicity in floral opening within a head. Asynchronicity is hypothesized to be an adaptation favouring smaller pollen vectors (Kenrick 2003; Stone *et al.* 2003). Given that flowers of subspecies saligna open asynchronously this suggests that small insects may be the primary pollen vector.

Female sterility within a flower head was observed to occur in all subspecies of *A. saligna* at a mean rate of 10 %

of flowers within a flower head. Female sterility has been observed in other species of *Acacia* as a highly variable and plastic character influenced by the environmental conditions experienced by the plants during floral development (Kenrick 2003; Sedgley 1989). There is insufficient information regarding environmental conditions at the study sites to attribute the variability in female sterility to any specific causes.

The pod set of *A. saligna* was low overall and showed significant differences between subspecies. This low pod set is comparable to results obtained by another study of the pod set of *A. saligna* conducted in the wheatbelt region of south-western Australia that found the proportion of inflorescences to set pods to be between 5.5 to 12.5% (Gaol & Fox 2002).

The domestication of *A. saligna* will necessarily involve the selection and cultivation of improved cultivars of the species. This work has identified a number of characters that will potentially make controlled crossing in *A. saligna* practically challenging, notably, the flower heads of *A. saligna* are small and dense, flowering is asynchronous, and it is difficult to maintain the viability of pollen in storage. Furthermore, compound pollen grains, of variable viability, and comparatively low seed-set mean that controlled crossing will provide low returns. The findings of this work suggest that using open pollination techniques may be more practical and cost effective than controlled crossing. The study has also shown that differences in certain aspects of reproductive biology may exist between the subspecies of *A. saligna*, particularly in terms of reproductive output. More work will be needed to explore these differences.

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Clonal *Santalum album* growth, oil content and composition on different hosts and at different locations

J A McComb

School of Biological Sciences and Biotechnology,
Murdoch University, Murdoch, WA, 6150
✉ jmccomb@murdoch.edu.au

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Abstract

The growth of clonal *Santalum album* on different hosts was assessed over 14 years at three different locations in Western Australia (Kununurra, Carnarvon and Geraldton). In trial one, two clones (K7 and LS) were grown on *Melaleuca* and *Azadirachta indica* as hosts at the three localities. Sandalwood growth was good at Kununurra and Carnarvon (increment of diameter at BH 0.8cm pa.), but poor at Geraldton. Heartwood was first detected in year 10 at Carnarvon. No heartwood formed at Geraldton up to year 14. Oil concentration in heartwood was highest at Kununurra (4.4% for the base of clone K7 with 65% santalols), and composition was similar to that published for juvenile trees growing in India. In the tallest trees, heartwood was present to 4m height in trunks, with only small differences between oil concentration and composition at different heights in the trunk.

In trial two, in which one clone (R32) was compared when grown at Kununurra with each of *Acacia mangium*, *Cassia siamea* or *Dalbergia sissoo* as a host, growth was best on *D. sissoo* and oil concentration highest using *A. mangium* as a host.

There was a range in growth and oil concentration within clones and improved silvicultural techniques are required if plantations are to benefit from the more uniform product theoretically possible from clonal material.

Keywords: *Santalum album*, oil content, oil composition, clones

Introduction

Santalum album L. (Indian sandalwood) an evergreen tree growing to 18 m, is prized for its aromatic heartwood. Although use of sandalwood from natural stands can be traced back 2,300 years in India (Srinivasan *et al.* 1992), there is some evidence it was introduced from Timor (Brand 1993, Fox *et al.* 1995). *S. album* grows in forests on the Indian peninsula, highland regions of eastern Indonesia and in Australia in scattered locations along the northern coast. It is cultivated in plantations in India, Indonesia, Australia and elsewhere (Radomiljac *et al.* 1998). World sources of sandalwood are declining rapidly (McKinnell 1993; Radomiljac *et al.* 1998) with significant harvest of plantation-grown timber yet to become available to the market. In India, poaching, adverse environmental conditions and spike disease are depleting sandalwood reserves while in Indonesia reserves are threatened by fire, grazing, and regulations that disadvantage the owners of the land on which sandalwood grows.

Seedlings of sandalwood are variable in growth rate, disease resistance, the age at which heartwood is initiated, and the content and quality of the oil (Srinivasan *et al.* 1992). The age at which trees initiate heartwood varies markedly with genotype and habitat. For example, Haffner (1993) reported that trees growing in West Timor ranged in age from 14–46 years for initiation of heartwood, while in India, heartwood initiation is generally expected within 10–13 years (Rai 1990). Also, the oil concentration and composition changes as trees mature. Young trees (about 10 years old, diameter >16 cm) have 0.2–2.0% oil, 60–85% santalols and higher santalyl acetate and santalenes than mature trees (30–50 years old, diameter ~32cm, 2.8–5.6% oil, ~90% santalols) (Srinivasan *et al.* 1992). It is the high level of the sesquiterpene alcohols α- and β-santalol in mature trees of *S. album* that is responsible for aroma and quality (Anonis 1998). In mature wood there is usually 50–60% α-santalol and 20–30% β-santalol with the latter contributing most to the typical sandalwood odour (Brunke and Hammerschmidt 1988).

In *S. album*, oil concentration is highest in roots and the butt of the tree, then decreases in the trunk and branches. The percentage of santalols decreases only slightly from the roots to the branches (Jayappa *et al.* 1981; Shankaranarayana & Pathasarathi 1987). In contrast, in *S. spicatum* there is a sharp change in oil composition at the root/shoot junction with α-and β-santalol both dropping markedly in concentration from the root to the trunk, and the concentrations of epi-α-bisabolol and t,t-farnesol increasing sharply (Piggott *et al.* 1997; Moretta 2001; Moretta *et al.* 2001).

Trees grown in different geographic areas are known to vary in oil content and composition, but it is difficult to disentangle genotypic differences and attribute specific differences to particular environmental conditions

(Jayappa *et al.* 1981). However, it has been suggested that trees which have higher rainfall and grow faster have a lower proportion of heartwood than those that grow more slowly due to water shortage. However hard data, particularly for plants given different management regimes on the same site, appear to be lacking (Applegate *et al.* 1990; Kealley 1991; Loneragan 1990).

Therefore, cloning superior trees appears a powerful tool to increase the productivity of plantations. Clones of superior trees were shown to have a faster growth rate and greater heartwood formation than seedlings, and after seven years, the selected clones showed measurements of girth and heartwood formation comparable to 50 year old trees (Lakshmi Sita 1991).

This paper reports on the effect of different hosts, and growth in different environments on oil content and composition of *S. album*.

Materials and Methods

Sources of plant material

Sandalwood clones

Clone R32 was raised from a four-year-old tree, grown from seed from Tangali, India, collected by Peter Richmond. (In 1990, it was one of the best trees on the CALM Arboretum in Kununurra, which was established in 1986.) Clone K7 was raised from a seedling from a tree grown at Kununurra in trials planted by Ian Richmond and Jen McComb. The provenance was Royalapad, India, and the tree was selected for good growth at year three in the Kununurra trial. Clone LS was from a tree grown in Lesmurdie (WA) from unselected seed.

Host plants

Plants of the temporary hosts *Sesbania formosa* or *Vigna unguiculata*, and of final hosts *Cassia siamea, Acacia mangium and Dalbergia sissoo*, were raised from seed. Later plantings of infill hosts were also raised from seed for planting in 1997 and 1988 at Carnarvon (*Cathormion umbellatum* a woody legume native to the Kununurra area) and at Geraldton (*Acacia acuminata* (jam)).

A clonal line of *Azaderachta indica* (neem) was selected from shoot cultures of seedlings grown in vitro. *Melaleuca quinquenervia* was from cuttings of a single genotype and was purchased from Zanthorrea Nursery. *M. leucadendra* was grown from cuttings in Kununurra.

Tissue culture protocols

Stem callus was induced from nodal sections of *S. album* trees cultured on solid Murashige & Skoog (MS) (1962) medium with 2.26 μM 2, 4-dichlorophenoxyacetic acid (2,4-D). Kinetin (2.23 μM) and indole acetic acid (5.71μM) were used in MS medium for callus proliferation and embryo induction. Embryogenic callus was transferred to medium with gibberellic acid (GA_3) (2.89 μM) for embryo maturation. Fully formed embryos were transferred to White's medium (Singh & Krikorian 1981) lacking plant growth regulators. Plantlets with both root and shoot apices growing were transferred to filter paper bridges dipping into liquid White's medium without sugar or growth regulators. All solid media contained 20 g L^{-1} sucrose, 2.5 g L^{-1} Gelrite and 2 g L^{-1} agar and had a pH of 5.7. Cultures were grown in polycarbonate tubs 7.5 X 7 cm and incubated at 25° C under constant light (30–40 μmol.m^{-2}.s^{-1}).

A. indica shoot multiplication was on MS with 2.5μM BAP, and rooting on 1/2 MS with 5μM IBA. Well grown rooted plants were potted and grown on in a glasshouse at Murdoch University. When sandalwood plants were 100 mm tall they were provided with an *Alternanthera* species as a pot host. Sandalwood was planted in the field when six months out of tissue culture (except for the replanting of Kununurra) and hosts were of a similar age.

Trial designs and assessments

There were two experiments. The first examined the effect of environment, and clones were planted at Geraldton, Carnarvon and Kununurra. The climate, soil and planting years at each site are described in Table 1. The second trial was at Kununurra and examined the effect of different hosts.

Table 1

Location, climate and soil at the three trial sites for sandalwood in Western Australia.

Location	Lat/Long	Mean rainfall (mm)/ irrigation	Soil type	Mean summer and winter daily maximum and minimum (lowest minimum, highest maximum) (°C)	Year planted
Frank Wise Research Station Kununurra	128 ° 44'E 15° 46'S	816 & flood irrigated	Cununurra clay	25.4–35.5 (47.8) 16.2–30.9 (7.9)	1991 replanted 1993
Dept of Agriculture Research Station Carnarvon	113 ° 66'E 24 ° 88'S	250 & drip irrigated	Sand to sandy loam, Gascoyne association light series	23.2–35.5 (49.2) 10.7–23.4 (1.1)	1991
40k N of Geraldton	114 ° 70'E 28 ° 78'S	425 & drip irrigated up to 3 times a week in years 1–5 then infrequent	Sandy clay	17.5–31.0 (46.0) 9.6–20.3 (2.0)	1991

Trial 1: The effect of environment: Trials of clones of S. album at Geraldton, Carnarvon and Kununurra

The two clonal lines of *S. album* were planted on similar final hosts at Kununurra, Carnarvon and Geraldton. In Kununurra, a temporary host (*Sesbania formosa*), was planted 1m from the sandalwood, and in Carnarvon *Vigna unguiculata*. No temporary host was used in Geraldton. Hosts and sandalwood were planted at the same time. The pigeon pea plants died within 12 months and the *Sesbania* within three years and are not considered further. At Carnarvon, it became evident over time that the sandalwood were also parasitising a roadside verge row of *Casuarina cunninghamiana*, as adjacent to the sandalwood trial the *Casuarina* heights were significantly reduced. At Geraldton, the tallest plants were 10 m from two mature native *Acacia acuminata*.

The permanent hosts were *Azadirachta indica* (syn. *Melia azadirachta*) neem, and *Melaleuca*. *M. quinquenervia* was used at Geraldton and Carnarvon and *M. leucadendra* at Kununurra. At Carnarvon and Geraldton, sandalwood and hosts were planted in the same row at 2 m spacing in the sequence neem, *Melaleuca quinquinerva*, sandalwood, *Melaleuca*, neem, sandalwood *etc*. At Kununurra, the sandalwood were planted on mounds, with plants 2 m apart in rows, with 2.5 m between (initially with the *Sesbania* 1m from each sandalwood), with the alternate rows being hosts in the row sequence *Melaleuca leucadendron*, sandalwood, neem, neem, *Melaleuca*, sandalwood *etc*. At Geraldton, after death of the neem an attempt was made to infill hosts by planting *Acacia acuminata* in 1997 and on the death of these plants this was repeated in 1998. These trees grew to 1–2m and are not considered further. At Carnarvon, *Calhornion umbellatum* was used to infill gaps. These had little impact: only 2 plants (12.5%) survived to 2005 reaching a mean height of 2.1m, and are not considered further.

At Carnarvon and Geraldton, there were four replicates of five plants of sandalwood clones K7 and LS. At Kununurra, there were three replicates of 10 plants of sandalwood clone K7.

Trial 2: The effect of host species: Comparison of sandalwood growth on three hosts at Kununurra

Clone R32 was planted on three final hosts *Acacia mangium*, *Cassia siamea* or *Dalbergia sissoo* in 1993. The field layout was as above with sandalwood provided with *Sesbania* as a temporary host. There were five rows of five sandalwood, alternating with rows of one of the three hosts and each host area was surrounded by a double row of the same host. Hosts and sandalwood were planted at the same time.

Initial, larger trials including all the sandalwood clones were planted at Kununurra in 1991 but were destroyed by corellas. Additional plants kept in pots for 18 months were used to set up the smaller trials outlined above in 1993.

Assessment of survival, growth and oil presence

Survival, height and trunk diameter were monitored at intervals. From year six onwards some of the larger sandalwood trees at Carnarvon and Geraldton were assessed for the presence of heartwood by drilling a 3 mm diameter hole at the base of the tree and testing the burned wood in the drill bit for the distinctive sandalwood odour.

Harvesting and oil analysis

In 2005, all remaining trees were harvested from the Kununurra plots and half of the trees from the Carnarvon plot. No trees were harvested at Geraldton. The trunk of each sandalwood was cut at 100 mm from the ground, then the soil around the tree was excavated and the remaining trunk and roots pulled from the ground. From each tree, a basal disk or "biscuit" of 30–50 mm in thickness was cut at ground level. In the comparison of clone R32 on different host species, from five of the tallest trees on each host, sample wood disks were cut both at the base level and at 1.5m height. In the comparisons of different clones at the same site, and of the same clone growing at different locations, for the K7, trees at Kununurra and for K7 and LS at Carnarvon, 4–5 trees were harvested at the base and at 1.5m height. A further analysis of the change in oil composition with position in the tree was carried out using one LS ramet and two K7 ramets from Carnarvon and two K7 ramets from Kununurra. From these, biscuits were cut at 20 cm intervals (to 1.6m) then 40cm intervals from the base to the top of the heartwood and from the base to the depth of roots where heartwood ceased. Tree diameter and percentage of area that was heartwood were assessed from the biscuits.

From each biscuit containing heartwood, a central section of 10 mm by 30 mm was marked. Biscuits without heartwood were not included. These sections were then cut out of the biscuits using a bandsaw and roughly powdered using a hand-held grinder. The ground samples were then processed and analyzed for oil concentration and composition, using a gas chromatography flame ionization detector (GC-FID) and a mass-selective detector (GC-MS), at Australian Botanical Products (ABP), Hallam, Victoria. The methods used by APB to extract the sandalwood oil and analyze the oil were similar to the methods described in Brand *et al.* (2007).

In summarising the data, values of zero for oil concentration (%) were included in the means of % oil concentration, but the values for α-and β-santalol were only included in the analysis when oil concentration was above 0.1%

Results

Trials of clones of *S. album* LS and K7 at Geraldton, Carnarvon and Kununurra

Survival and growth of the hosts at the three locations

At Geraldton, all neem died except for one plant that survived for 7 years but only reached 1m in height (Fig. 1). At Geraldton, neem was winter deciduous, while it was evergreen at the other locations. Melaleucas at Geraldton had 100% survival, reaching 5.7m after 10 years and thereafter showing no increase in height (Fig. 2). The neem grew well at Carnarvon with the best trees reaching a height of 9–10m, but over time they were damaged or killed by the sandalwood, reducing the final mean height to 4.2m and survival to 60% (Fig. 1).

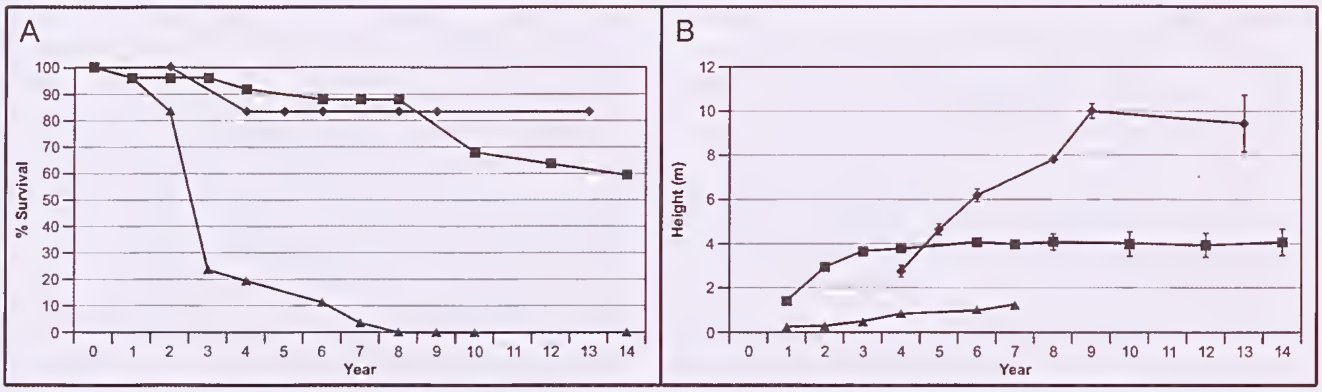

Figure 1. Survival (A) and mean growth (B) of neem at Geraldton ▲, Carnarvon ■ and Kununurra ◆. Bars on height data indicate standard errors.

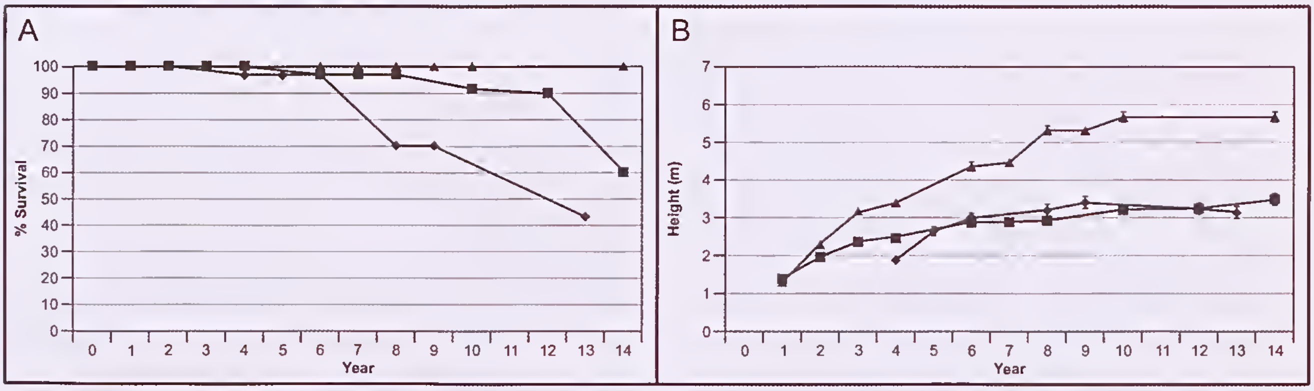

Figure 2. Survival (A) and mean growth (B) of *Melaleuca* at Geraldton ▲, Carnarvon ■ and Kununurra ◆. Bars on height data indicate standard errors where large enough to display.

Melaleuca showed 60% survival but trees were generally unhealthy with many dead twiggy branches and a final height of only 3.5m (Fig. 2). At Kununurra, neem survival was 83% and trees reached 10 m after nine years and then did not increase in height (Fig. 1). *Melaleuca* had 43% survival. It grew to 3m after six years which was its maximum height (Fig. 2).

Sandalwood survival and growth

Final survival of *S. album* K7 at Kununurra was poor at 33% with most deaths occurring at age 4–6 years, compared with 80–90% survival at Geraldton and Carnarvon (Fig. 3). Clone LS gave only 41% survival at Geraldton compared with 60% at Carnarvon, where most losses were at establishment (Fig. 3).

At Geraldton, sandalwood Clones K7 and LS grew at comparable rates with little increase after 10 years (Figs. 3 and 4). The tallest trees were 5.5m tall (K7) and 4.4m (LS). The mean height of LS was greater than that for K7 but the difference was not significant. Trees at Geraldton were significantly shorter than those at the other two

Table 2

Range of sandalwood heights and oil concentration within clones. Height data are for all surviving trees at year 14, oil concentration is for 4-5 of the tallest trees of each clone.

Location	Clone	Height range (m) Minimum	Maximum	n	Oil % concentration range Minimum	Maximum	n
Kununurra	R32*	2.2	7.0	10	0.02	3.85	5
	K7	3.2	8.2	8	2.95	5.71	4
Carnarvon	K7	3.9	9.6	19	0.65	4.03	5
	LS	3.9	9.6	12	2.47	3.55	5
Geraldton	K7	1.4	5.4	14	na	na	
	LS	1.5	4.4	8	na	na	

*on *A. mangium*

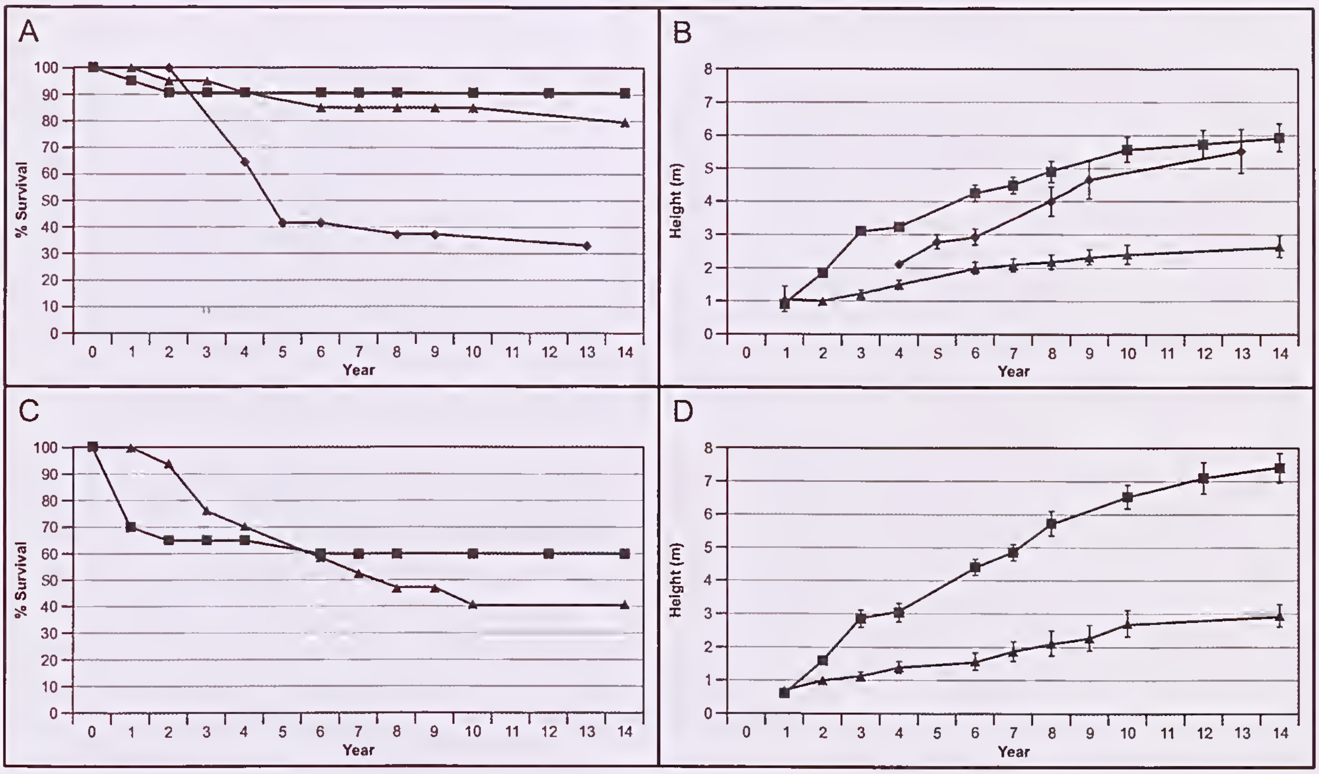

Figure 3. Survival and mean growth of two *S. album* clones K7 (A and B) and LS (C and D) at Geraldton ▲, Carnarvon ■ and Kununurra ◆. Bars on height data indicate standard errors where large enough to display.

Table 3

Sandalwood trunk mean diameters (overbark) at the base and 1.5m height in year 14 for sandalwood clones K7 and LS grown on *Melaleuca* and neem at three locations.

Location	Clone K7 diameter (cm) (SE) Base	1.5m	No.	Clone LS diameter (cm) (SE) Base	1.5m	No.
Geraldton	6.8 (0.91)	3.5 (0.60)	16	6.6 (1.00)	3.7 (0.70)	7
Carnarvon	16.7 (1.02)	10.9 (0.87)	19	19.3 (1.62)	13.6 (1.22)	12
Kununurra	13.2 (1.05)	10.2 (0.83)	8	na	na	

na: not available

sites. Growth of sandalwood was very similar at Carnarvon and Kununurra with clone K7 growing faster at Carnarvon than Kununurra. At Carnarvon, clone LS had significantly greater mean height than K7 (7.4 compared to 6.0m) (Fig. 4). The range in plant heights of individual clones was considerable (Table 2).

There was little difference between trunk diameters of K7 trees grown at Carnarvon or Kununurra, while those at Geraldton were significantly narrower (Table 3). At Carnarvon, clone LS had greater diameter than K7, but this was not the case at Geraldton. The mean annual increment of diameter at breast height in the trials at Carnarvon and Kununurra was around 8 mm.

Table 4

Mean trunk diameter (overbark) and percentage heartwood for all sandalwood trees from which oil was analysed. SE of means are shown in parenthesis. (All trees had heartwood at the base; only samples with heartwood are included in the data for 1.5m)

Host	Base Trunk diameter (cm)	Heartwood (%)	n	1.5m Trunk diameter (cm)	Heartwood (%)	n
Clone K7 at Kununurra						
Melaleuca & neem	13.9 (0.95)	42.9 (5.1)	7	11.5 (0.91)	30.9 (13.07)	4
Clone K7 at Carnarvon						
Melaleuca & neem	14.7 (1.29)	46.9 (4.49)	9	10.7 (0.74)	29.4 (2.39)	5
Clone LS at Carnarvon						
Melaleuca & neem	18.1 (1.36)	43.7 (4.13)	7	14.1 (1.48)	27.8 (8.90)	5
Clone R32 at Kununurra						
Acacia mangium	13.3 (1.19)	17.5 (5.00)	13	9.2 (0.97)	6.2 (4.98)	4
Dalbergia sissoo	13.0 (0.95)	28.6 (3.00)	15	9.9 (0.83)	3.8 (1.64)	4
Cassia siamia	8.6 (0.33)	34.9 (4.00)	16	6.2 (–)	17.7 (–)	1

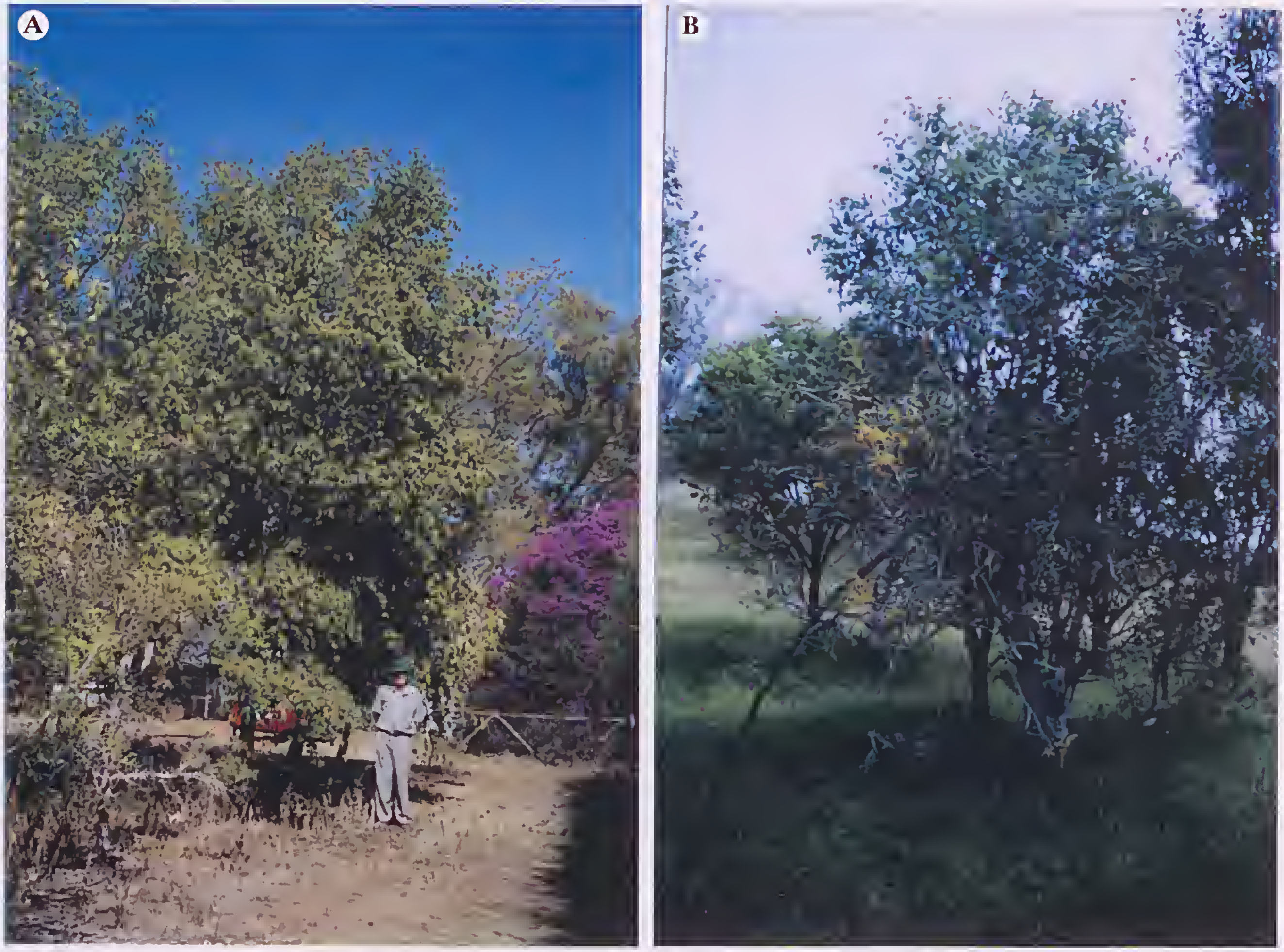

Figure 4. A. *S. album* at Carnarvon in year 12. The first tree in the row is a 6m neem, then a K7 sandalwood (8.8m) and an LS sandalwood (9.6m). B. *S. album* at Geraldton in year 15. From left two sandalwood K7 clones (4.4 and 5.4m tall) growing next to *Metaleuca quinquenervia* with an infill *Acacia acuminata* in the foreground.

Sandalwood on three host species at Kununurra

Survival and growth of hosts

C. siamea had almost 100% survival until year nine, but dropped to 56% by year 13 (Fig. 5). Growth plateaued after year nine at 11m. Survival of *A. mangium* and *D. sissoo* fell away steadily until at year 15 there were only 21% of both species alive (Fig. 5).

Survival and growth of sandalwood R32

Only 21–28% of the clone R32 sandalwood on the three different hosts survived with most deaths being over the first four years (Fig. 5). Sandalwood trees reached 4.0 m on *C. siamea*, 5.0 m on *A. mangium*, and 6.1 m on *D. sissoo*. Trunk diameters of trees that were used in the oil analysis, were similar between sandalwood grown with *D. sissoo* and *A. mangium*, both at the base and at 1.5 m, but were significantly smaller on *C. siamea* (Table 4).

Plant form

Clones K7 and LS required light pruning in the first five years to attain a long straight bole. The clone R32 had poor form (almost plagiotropic) and required heavy pruning in years 1–5 to achieve an upright tree. Inadequate weed control within rows meant that many trees developed a trunk bent towards the light in the mown alleyways between the rows). The roots of the excavated trees at both Kununurra and Carnarvon were very shallow and thin (Fig. 6).

Heartwood formation and oil analysis

Effect of site and clone on heartwood (Clones K7 and LS)

Heartwood was first detected in the larger trees at Carnarvon when drilled in year 10. No heartwood was apparent from drilling in Geraldton up to year 14. There was no significant difference between the mean percentage heartwood between clones K7 and LS at Carnarvon, or between K7 when grown at Carnarvon and Kununurra (Table 4). When the oil concentration and composition of K7 grown at Kununurra and Carnarvon were compared, the concentration at Kununurra was much higher from the basal samples, but not at the 1.5m height, and the α- and β-santalol were similar in both locations for samples at the same heights (Table 5). There was a wide range in percentage oil concentration within clones (Table 2). A detailed analysis of the oil constituents is shown in Table 6, with the most important components being α- and β-santalol, bergamotol and epi- β-santalol.

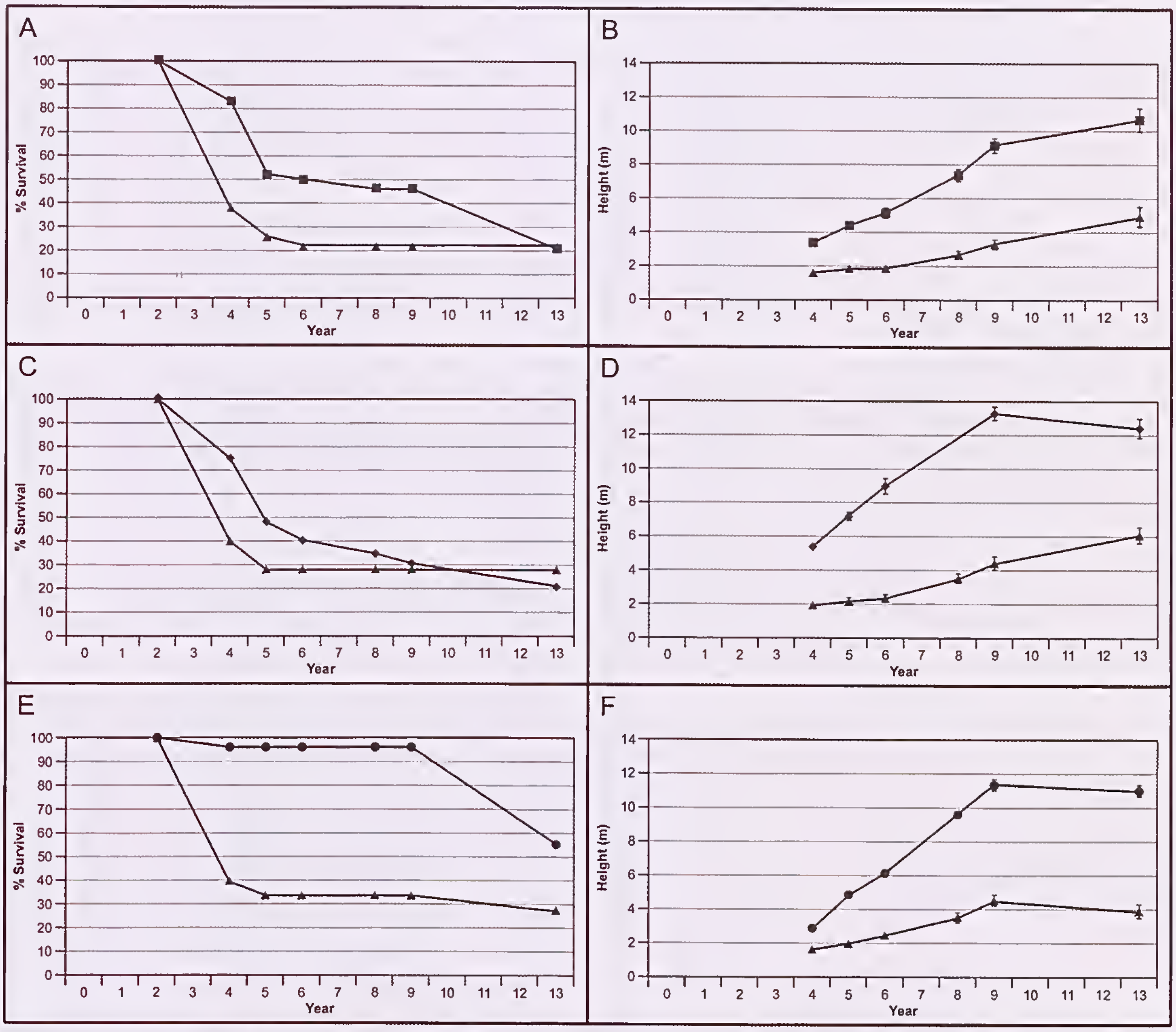

Figure 5. Survival and mean growth of sandal clone R32 ▲ on *Acacia mangium* ■ (A and B), *Dalbergia sissoo* ◆ (C and D) and *Cassia siamea* ● (E and F) at Kununurra. Bars on height data indicate standard errors where large enough to display.

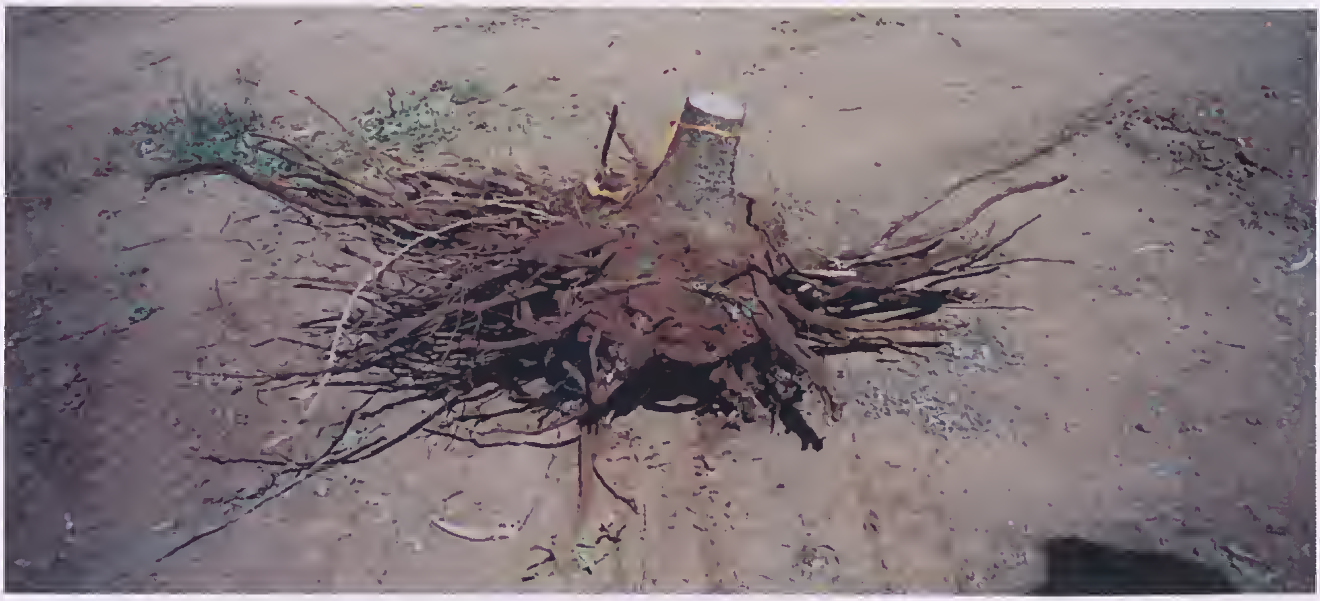

Figure 6. An excavated *S. album* root system at Carnarvon

Table 5

Mean % oil concentration and % α- and β-santalol for clone K7 grown at two localities, Kununurra and Carnarvon.

Location	Oil concentration (%) (SE)		n	α-santalol (%) (SE)		n	β-santalol (%) (SE)		n
Base									
Kununurra	4.38	(0.727)	4	44.05	(1.071)	4	21.91	(0.587)	4
Carnarvon	1.90	(0.590)	5	43.66	(0.703)	5	20.60	(1.138)	5
1.5m									
Kununurra	1.16	(0.653)	4	43.3	(0.704)	2	20.6	(1.139)	2
Carnarvon	1.23	(0.718)	5	41.83	(0.706)	3	19.56	(0.335)	3

A comparison of the two clones growing on the same site (K7 and LS at Carnarvon) showed the two clones having similar concentrations at the base. At 1.5m, there was the same oil concentration in both clones with K7 being slightly higher in β-santalol (Table 7).

Comparison of oil concentration and composition at different heights in the roots and trunk showed only small differences between sections (Table 8). A comparison of clone K7 at Carnarvon and Kununurra indicates that concentration was higher at Kununurra at most positions in the tree but that α-and β-santalol were consistently higher at Carnarvon (Table 9).

Table 6

Mean oil concentration and composition from the base samples of K7 trees from Kununurra and Carnarvon (n=9).

	Mean concentration (g/kg) (SE)	Composition (%) (SE)
α-santalene	0.34 (0.057)	1.26 (0.134)
α-trans-bergamotene	0.06 (0.011)	0.23 (0.27)
epi-β-santalene	0.36 (0.061)	1.32 (0.123)
β-santalene	0.50 (0.083)	1.88 (0.211)
α-santalol	13.18 (2.761)	43.83(0.577)
cis-α-trans-bergamotol	1.85 (0.425)	5.89 (0.266)
epi-β-santalol	1.03 (0.222)	3.41 (0.097)
β-santalol	6.53 (1.445)	21.18 (0.686)
cis-nuciferol, curcumen-12-ol isomer	0.22 (0.069)	0.65 (0.133)
cis-lanceol	0.28 (0.114)	0.74 (0.173)
Total (g/kg)	30.02 (6.126)	80.40 (1.118)
Concentration (%)	3.00 (0.613)	

There was some evidence that faster growing trees had less oil content as the correlation between height growth and oil concentration (%) for LS was (r= -0.73) and for K7 (r= 0.11). The clonal lines showed considerable variation in oil concentration within the same genotype (Table 3).

Table 8

Mean % oil concentration and α- and β-santalol for all clones at different positions from the root to the height where heart wood ceased.

Height (cm)	Oil concentration (%) (SE)	α-santalol (%) (SE)	β-santalol (%) (SE)
–80	0.06 (–)		
–60	2.41 (2.340)	36.05(–)	16.96 (–)
–40	1.81 (1.770)	41.96 (–)	20.92 (–)
–20	1.85 (0.838)	42.64 (2.069)	21.01 (1.072)
0	2.73 (0.870)	44.97 (0.695)	20.76 (1.019)
20	3.64 (1.191)	44.15 (1.149)	21.30 (0.850)
40	2.43 (0.846)	44.38 (0.260)	20.74 (1.297)
60	1.60 (0.490)	42.21 (3.380)	20.34 (0.681)
80	1.68 (0.486)	43.70 (1.572)	20.11 (0.770)
100	2.58 (0.631)	45.18 (0.571)	21.32 (0.429)
120	2.79 (0.697)	44.90 (0.711)	20.77 (0.684)
140	2.47 (0.711)	41.69 (1.566)	19.08 (0.971)
160	1.37 (0.617)	43.50 (1.332)	20.08 (0.517)
180			
200	0.84 (0.643)	39.16 (7.830)	17.74 (4.540)
220			
240	1.08 (0.915)	20.22 (12.203)	9.15 (6.613)
260			
280	1.57 (1.460)	31.03 (11.455)	13.79 (6.610)
300			
320	2.6 (–)	40.36 (–)	20.11 (–)
340			
360	2.41 (–)	38.4 (–)	18.20 (–)
380			
400	2.16 (–)	45.52 (–)	20.22 (–)

Table 7

Mean % oil concentration and α- and β-santalol for clones K7 and LS grown at Carnarvon.

Clone	Oil concentration (%) (SE)		n	α-santalol (%) (SE)		n	β-santalol (%) (SE)		n
Base									
LS	2.88	(0.200)	5	43.04	(2.274)	5	18.97	(0.851)	5
K7	1.90	(0.590)	5	43.66	(0.703)	5	20.60	(1.138)	5
1.5m									
LS	1.23	(0.311)	5	37.98	(2.767)	5	15.40	(1.372)	5
K7	1.54	(0.837)	4	41.83	(0.706)	3	19.56	(0.335)	3

Table 9

Mean % oil concentration and α- and β-santalol for clone K7 trees at Kununurra and Carnarvon at different positions from the root to the height where heart wood ceased (two trees were assessed from each location).

Height (cm)	Oil concentration (%) Carnarvon	 Kununurra	α-santalol (%) Carnarvon	 Kununurra	β-santalol (%) Carnarvon	 Kununurra
–80	0.06 (–)		0.00 (–)		0.00	
–60	0.07 (–)		0.00 (–)		0.00	
–40	0.03 (0)	0.06 (–)	1.72 (1.72)	1.57 (–)	0.00	0.33 (–)
–20	2.39 (0.46)	0.04 (0.02)	41.21 (2.59)	18.38 (0.24)	20.33 (1.43)	9.20 (0)
0	1.07 (0.42)	4.51 (1.20)	44.22 (1.58)	32.53 (1.09)	19.65 (1.75)	15.46 (0.69)
20	1.51 (0.38)	6.50 (0.49)	43.33 (2.80)	31.95 (2.00)	20.50 (1.06)	15.80 (0.86)
40	1.15 (0.30)	4.22 (1.31)	44.26 (0.59)	31.98 (0.40)	19.57 (2.16)	15.65 (0.03)
60	1.20 (0.26)	1.64 (1.38)	45.73 (1.68)	30.72 (8.21)	21.73 (0.60)	15.34 (0.78)
80	0.80 (0.29)	2.17 (1.00)	40.89 (3.25)	31.21 (0.85)	19.39 (1.99)	14.93 (0.22)
100	1.42 (0.44)	4.03 (0.39)	44.33 (0.42)	32.34 (1.30)	21.84 (0.32)	15.87 (0.08)
120	1.39 (0.62)	4.10 (0.87)	45.18 (0.45)	32.32 (1.93)	20.41 (1.98)	15.37 (0.65)
140	1.70 (0.87)	2.58 (1.68)	40.15 (4.40)	30.16 (0.46)	18.62 (2.60)	14.18 (1.52)
160	1.85 (1.60)	1.02 (0.93)	41.31 (0.83)	27.46 (13.62)	19.31 (0.40)	13.04 (6.44)
180						
200	0.38 (–)	0.03 (–)	31.33 (–)	1.07 (–)	13.20 (–)	0.00 (–)
220						
240	0.12 (–)		13.42 (–)	3.32 (–)	4.47 (–)	1.67 (–)
260						
280	0.11 (–)		0.00 (–)		7.18 (–)	

Table 10

Mean % oil concentration and % α- and β-santalol for clone R32 grown on three hosts at Kununurra. In summarising the data on % oil concentration, values of zero were included, but the values for α- and β-santalol were only included for oil concentrations above 0.1%.

	Host *Dalbergia sissoo*	n	 *Acacia mangium*	n	 *Cassia siamea*	n
Base						
Oil concentration%	0.38 (0.248)	5	2.17 (0.783)	5	0.03 (0.006)	5
α-santalol %	19.99 (6.045)	4	37.06 (2.648)	4	–	0
β-santalol %	9.25 (2.975)	4	17.12 (0.933)	4	–	0
1.5m						
Oil concentration%	0.18 (0.093)	5	0.51 (0.408)	5	0.09 (0.089)	5
α-santalol %	15.36 (8.944)	4	27.44 (6.938)	2	28.07 (0)	1
β-santalol %	7.25 (4.608)	4	13.14 (3.846)	2	13.14 (0)	1

Effect of host on heartwood in Clone R32

At Kununurra, the percentage heartwood was similar in trees grown on *A. mangium* and *D. sissoo* (Table 4). Sandalwood trees growing on *A. mangium* as a host had a higher oil concentration, and α- and β-santalol at both the base and at 1.5 m (Table 10). Sandalwood trees grown on *C siamea,* were smaller than those on *A. mangium* or *D. sissoo.* The oil concentration was negligible at the base of all five trees sampled and only one tree had heartwood at 1.5 m. although this single sample had a high per cent heartwood and similar α-and β-santalol to the trees on *A. mangium* (Table 10).

Discussion

The range of locations used in these clonal trials showed that the warmer, frequently irrigated Carnarvon site and Kununurra with monsoonal rains and winter irrigation were suitable for plantations of *S. album,* but the colder, less frequently watered Geraldton site was not. The annual increment (at breast height) in the trials at Carnarvon and Kununurra was 8 mm pa, which was in the middle of the range reported from India (3–16 mm pa, Venkatesan 1980). The trees in Geraldton averaged only 2.3 mm pa.

Clones did not give any more even growth or oil concentration than would be expected from seedlings but improved silviculture methods might reduce the variability. There were small but significant differences in clone height and diameter with clone LS being superior to clone K7 and possibly clone R32, although this was not grown at the same site. It was necessary to prune all genotypes to achieve an unbranched bole, but clone R32 was initially particularly poor, with thin weak stems, presumably an occasional undesirable effect of cloning through somatic embryogenesis in vitro.

Oil concentration and composition obtained from the 14-year-old trees matched published data from juvenile trees in India (Srinivasan *et al.* 1992). All trees from ramets of clones K7, LS and R32 had heartwood at the base after 14 years when grown at Carnarvon or Kununurra. A higher oil concentration and more valuable composition is expected in heartwood of older trees. The remaining half of the clonal trial at Carnarvon will provide useful information on oil in trees of different ages.

The 14-year-old trees had less oil concentration in roots compared to trunks, and the roots developed at this age were thin with significant oil concentration only to 20 cm depth (with traces to 80 cm depth). This is contrary to published data for mature Indian trees that showed the lateral roots with high oil concentrations (Srinivasan *et al.* 1992).

Oil content was higher in trees grown at Kununurra compared to Carnarvon, and one of the two clones at Carnarvon had a higher percentage of santalols. Data for 10 year old sandalwood trees grown at Kununurra (Jones *et al.* 2007), cannot be compared to those in this paper as the earlier data included sap as well as heartwood in the analysis.

Dalbergia sissoo, which provided dappled shade and was the tallest of the host species amongst the legumes tested using clone R32, supported the best growth of sandalwood while those hosted onto *A. mangium* showed highest oil concentration. The low survival of hosts was a problem as there were very few host trees of *A. mangium* or *D. sissoo* remaining at year 14. A higher host-to-parasite ratio may be necessary to improve long-term sandalwood survival and growth. It is important to establish permanent hosts in a ratio that will support mature sandalwood at plantation establishment as infill of hosts amongst existing sandalwood was difficult.

Melaleuca and neem were chosen as hosts in this experiment as it was thought that they would survive at all three locations. However, the winter temperatures at Geraldton proved unsuitable for neem. In any case neem is not recommended as a host as in some areas of northern Australia it has become an environmental weed from berries carried by birds to riparian zones (Grice 2002). Nitrogen fixing hosts appear beneficial as indicated by the better growth of sandalwoods at Geraldton that fortuitously attached to nearby wild *Acacia accuminata* and those at the Carnarvon trial next to the street planting of *Casuarina cunninghamiana*. Thus the potential for growth and heartwood production is probably underestimated from the work using *Melaleuca* and neem as hosts, and this work suggests a higher host to sandal ratio might also be beneficial.

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Notes on ....

Range extensions for the Barking Gecko, *Nephrurus milii* (Squamata: Gekkonidae)

S A Thompson[1], G G Thompson[2] & G R Finlayson[1]

[1] Coffey Environments
PO Box 4223 Victoria Park 6979 Australia
✉ scott_thompson@coffey.com
✉ graeme_finlayson@coffey.com

[2] Edith Cowan University
Joondalup Drive, Joondalup, WA, 6027
✉ g.thompson@ecu.edu.au

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Abstract

Recent captures of *Nephrurus milii* increase its recorded geographical distribution, suggesting the goldfields population extends further north than has previously been recorded and the Pilbara population is more widespread than has been earlier reported. The relative abundance of this species appears to differ appreciably across its range with the consequence that its presence could easily have gone undetected in the northern mid-west and Murchison regions and other sections of the Pilbara due to a lack of adequate survey effort.

Key words: disjunct populations, Western Australia, lizard

Introduction

Menz & Cullen (2006) recently reported a disjunct population of *Nephrurus milii* in the eastern Pillbara, near Packsaddle Range and commented on a sighting of a specimen by Michael Kearney at Handrai Pool in Karijini National Park. The Barking Gecko, *N. milii,* is known to occupy a variety of habitat types, including sand plains, woodlands, shrublands, rocky outcrops, breakaways and rubbish tip sites (Storr *et al.* 1990; Thompson & Thompson, 2006; Wilson & Swan, 2005). Until recently the known distribution of *N. milii* was across the southern half of Australia from Rockhampton, Queensland to Shark Bay Western Australia (Wilson & Swan, 2005). We report here on two recent captures that extend its geographic distribution north in the Goldfields and north-west in the Pilbara and comment on its highly variable abundance and the consequential implications for detecting the presence of this and other isolated populations.

Observations

On 15 January 2007 at about 2100hr, a Barking Gecko was observed at (WGS 84) 51 239592E, 7027514N, in an area about 30km south of Wiluna (Fig 1). This gecko was foraging in an open Mulga and chenopod scrubland. The specimen was vouchered with the Western Australian Museum (R166876). This record represents a range extension approximately 125km north of the commonly published range for *N. milii* in the Goldfields. A pit-trapping program (3360 bucket pit trap-nights, 3360 pipe pit trap-nights and 6720 funnel trap-nights) in the area during October 2006 and January 2007 failed to catch another specimen.

On 18 November 2008, a *N. milii* was caught in a pit-trap in the Hamersley Ranges, north-west of its known distribution ((WGS 84) 50 594731E 7553687N). This specimen was vouchered with the Western Australian Museum (R163638). It was caught in the floor of a gorge valley that was surrounded by steep rocky slopes. The valley floor was a gravelly-clay substrate vegetated with eucalypts and other small trees to about 2.5m over spinifex and small shrubs. This area had been trapped in March 2008 but the presence of *N. milii* was not detected. A trapping program in similar habitats in the vicinity of where this specimen was caught consisted of 9420 bucket pit trap-nights, 9420 pipe pit trap-nights and 21840 funnel trap-nights during March and November 2008.

In another recent trapping survey in an area approximately 170km north of Laverton, again on the periphery of *N. milii*'s goldfields distribution in spinifex

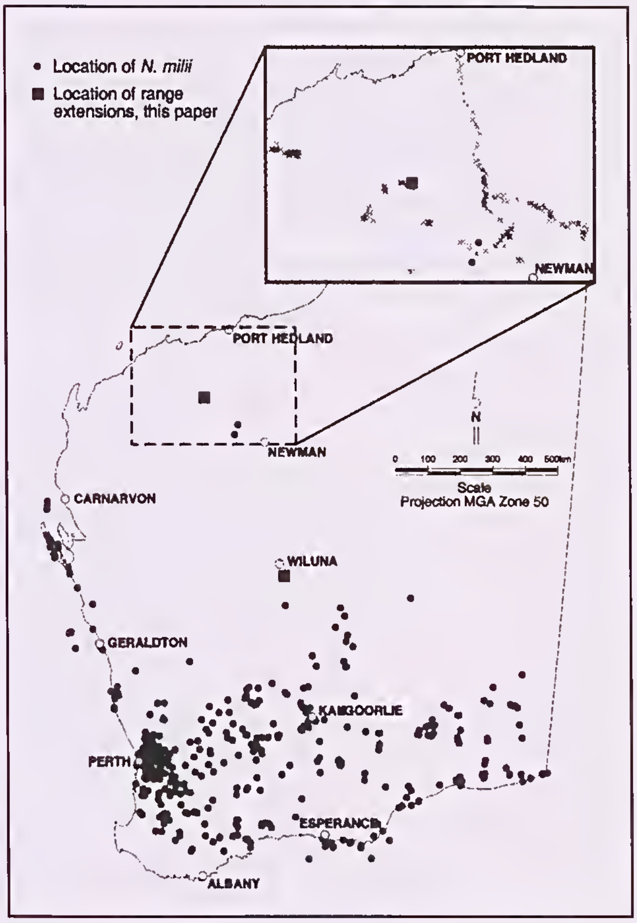

Figure 1. Capture locations for *N. milii*. Squares represents the two range extensions reported here, dots are other records of *N. milii* from the Western Australian Museum and crosses are survey sites in the Pilbara where *N. milii* was not caught.

vegetated areas, eucalypt woodlands over spinifex or shrubs and open mulga woodlands, no *N. milii* were caught using pit-traps (4032 bucket pit trap-nights, 4032 pipe pit trap-nights) and funnels (8064 funnel trap-nights), however, an individual was seen on the road during spotlighting ((WGS 84) 51 434150E, 6958000N). In another trapping program east of Ravensthorpe in a variety of habitats including eucalypt woodlands and heath, no *N. milii* were trapped using pit-traps (1680 bucket trap-nights, 1680 pipe pit trap-nights) and funnel traps (3360 funnel trap-nights), however a single individual was caught during hand searching ((WGS 84) 51 257581E, 6276324N).

Discussion

It is common to find isolated populations of reptile species within their published geographical distribution as few species are either evenly distributed across their geographic range or evenly distributed within their recognised preferred habitats. In some areas *N. milii* has a high abundance and is readily detected early in a trapping program, however, in other areas it appears to have a very low abundance and is rarely trapped. For example, during extensive fauna surveys around Ora Banda (Thompson & Thompson 2005; Thompson *et al.* 2003) 946 *N. milli* were caught in a diverse range of habitat types including mine waste dumps, rocky areas cover with spinifex, clay soils vegetated with tall eucalypts or low chenopod shrubs. Further analysis of the Ora Banda data shows that *N. milii* were significantly ($t_{4,7}$ = 4.07, $P < 0.05$) more abundant on rehabilitated waste dumps than in adjacent areas of undisturbed habitat. Mean capture rates in areas of undisturbed habitat were 3.33 per 1000 trap-nights compared with 18.2 per 1000 trap-nights in rehabilitated waste dumps. Higher numbers on waste dumps presumably reflected the abundance of crevices in this habitat and the ability of this species to rapidly colonise new areas after major disturbance (Thompson & Thompson 2007).

Elsewhere within *N. milii*'s geographic distribution its abundance appears to be low. Cowan & How's (2004) survey of Goongarrie Station during March 1979 and 2002, and October 1980 and 2001 recorded only nine individuals all of which were caught at a single site. This survey site is about 75km north of Ora Banda and is both well within the previously recorded geographic distribution for *N. milii* and contains habitat similar to that at the Ora Banda survey sites. During our trapping programs north of Laverton, south of Wiluna and east of Ravensthorpe no *N. milii* were caught in pit-traps or funnel traps, and they were only detected during spotlighting or hand-searching. It is therefore apparent that this species can be relatively rare within its distribution and could easily go undetected during a trapping program.

Although there have been numerous fauna surveys recently conducted in the Pilbara (296 survey sites; see insert in Fig 1; Biota Environmental Sciences 2002a,b,c; 2004; 2005a,b,c; 2008; Davis *et al.* 2005; Johnstone 1983; Ninox Wildlife Consulting 1985; 1992; Texasgulf 1979), many of which have been undertaken to support an environmental impact assessment for a mining development, *N. milii* has gone undetected until the Menz and Cullen (2006) capture.

The presence of a disjunct population of *N. milii* in the Pilbara raises the questions of how did they get there and how widely are they distributed in the region. Figure 1 indicates they are absent from the northern mid-west and inland Murchison regions. However, the area between Wiluna and the Pilbara has generally been poorly surveyed, so it is possible that *N. milii* are present in the area in low numbers and have gone undetected.

Range extensions often go unreported when detected during fauna surveys undertaken by environmental consultants. For example, in the northern Goldfields, Ninox Wildlife Consulting (1994) reported catching *N. milii* during its survey of a site close to our survey sites about 30km south of Wiluna, but did not comment about its distribution or suggest that it was a possible range extension in its report to the client. This maybe because the consultant is unaware the capture represents a range extension or consultants are too busy and do not see the information as being note worthy.

The recent capture of *N. milii* in the Pilbara raises issues to do with the intensity of fauna surveys undertaken to support environmental impact assessments. Remnant and isolated populations are generally recognised as being of conservation significance. Sometimes, there is an abundance of individuals in these isolated populations, whereas other times they are relatively rare. If they are rare, then the probability of detecting their presence with a low intensity survey effort is low. As the purpose of these surveys is to assess the potential impact of vegetation clearing and development on the fauna assemblage in an area, and in particular identify the presence of conservation significant species, there is a strong case for more intensive and comprehensive surveys being undertaken to ensure these isolated populations are detected.

Typically, the trapping effort undertaken by environmental consultants to assess the presence and relative abundance of small vertebrate fauna in a habitat is low and unlikely to catch most of the species present. Thompson *et al.* (2007) recommended for habitats that were species poor (*e.g.* 10-15 species), 70-100 individuals need to be caught to record about 80% of the species, and 90-200 individuals caught to record about 90% of the species. When a habitat contained approximately 35 species, then 100-280 individuals need to be caught to record 80% of the species, and 200-600 individuals will need to be caught to record 90% of the species, When the proportion of rare species in a habitat was high (*e.g.* high proportion of singletons and doubletons), then a higher trapping effort will be required.

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